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BIOLOGICAL BULLETIN

OF THE

Marine Biological Laboratory

WOODS HOLE, MASS.

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VOLUME XXIV.

WOODS HOLE, MASS.
DECEMBER 1912, TO MAY 1913

PRESS OF
THE NEW ERA PRINTING COMPANY
LANCASTER, PA.

2025

CONTENTS OF VOLUME XXIV.

No. 1. December, 1912.

Shull, A. Franklin. *The Influence of Inbreeding on Vigor in Hydatina senta* 1
Grave, B. H. *The Otocyst of the Pinnidæ* 14
Ruthven, Alexander G. *On the Breeding Habits of Butler's Gartersnake* 18
Allyn, Harriett M. *The Initiation of Development in Chætopterus* 21

No. 2. January, 1913.

Goldfarb, A. J. *Studies in the Production of Grafted Embryos* . 73
Pearse, A. S. *On the Habits of the Crustaceans found in Chætopterus Tubes at Woods Hole, Massachusetts* 102
Rawls, Elizabeth. *Sex Ratios in Drosophila ampelophila* 115

No. 3. February, 1913.

Boring, Alice M. *The Odd Chromosome in Cerastipsocus venosus* 125
Boring, Alice M. *The Chromosomes of the Cercopidæ* 133
Lillie, Frank R., and Just, E. E. *Breeding Habits of the Heteronereis Form of Nereis limbata at Woods Hole, Mass.* . . 147
Abbott, J. F. *The Effect of Distilled Water upon the Fiddler Crab* 169
Glaser, Otto. *On the Origin of Double-Yoked Eggs* 175
Foot, Katharine, and Strobell, E. C. *Preliminary Note on the Results of Crossing Two Hemipterous Species with Reference to the Inheritance of an Exclusively Male Character and its Bearing on Modern Chromosome Theories* 187

No. 4. March, 1913.

Pearl, Raymond, and Parshley, H. M. *Data on Sex Determination in Cattle* 205
Hartman, Frank A. *Variations in the Size of Chromosomes* . . . 226
Hartman, Frank A. *Giant Germ Cells in the Grasshopper* 239
Rau, Phil and Nellie. *The Fertility of Cecropia Eggs in Relation to the Mating Period* 245
Wilder, Inez Whipple. *The Life History of Desmognathus fusca* 251

No. 5. April, 1913.

Wilder, Inez Whipple. *The Life History of Desmognathus fusca* 293
Heilbrunn, Lewis V. *Studies in Artificial Parthenogenesis* 343
Cole, Leon J. *Experiments on Coördination and Righting in the Starfish* 362
Stocking, Ruth J. *A Note on the Spermatogenesis of Tenebrio molitor* 370

No. 6. May, 1913.

King, Helen Dean. *Some Anomalies in the Gestation of the Albino Rat (Mus Norvegicus albinus)* 377
Wilson, Edmund B. *A Chromatoid Body Simulating an Accessory Chromosome in Pentatoma* 392
Kepner, William A., and Taliaferro, William H. *Reactions of Amœba proteus to Food* 411
Fifteenth Report of the Marine Biological Laboratory 429

Vol. XXIV. *December, 1912.* *No. 1.*

BIOLOGICAL BULLETIN

THE INFLUENCE OF INBREEDING ON VIGOR IN HYDATINA SENTA.[1]

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Page
Introduction 1
Description of the experiments 1
The measure of vigor 2
Results of the experiments 4
Other evidence relating to vigor in inbreeding 6
Mendelian explanations of vigor 7
Loss of vigor not accounted for by the Mendelian explanations 10
A physiological explanation of vigor 10
Bibliography 12

INTRODUCTION.

In some experiments dealing with the inheritance of certain egg characters in the rotifer *Hydatina senta*, it has been necessary to inbreed the animals a number of times in succession. Evidence relating to the influence of inbreeding on vigor has thus been incidentally obtained. Since this part of the evidence from the experiments has no direct bearing on the inheritance of the egg characters in question, it is published here separately.

DESCRIPTION OF THE EXPERIMENTS.

All the experiments started from a single female, herself an F_1 from a cross. From her was bred a parthenogenetic line of 12 generations. Some of the females and males of this line were paired, and a considerable number of fertilized eggs obtained. When the fertilized eggs hatched, two of the earliest hatchers, one from a family in which a large proportion of the eggs hatched, one from a family in which only a few hatched were selected for further breeding. From these two females were bred two parthenogenetic lines, of four and ten generations, respectively. In each line some females were paired with males of the same line, and from one of the fertilized eggs of each lot a new parthenogenetic line was reared. The members of these were again inbred, and so on, six times in succession. There were thus obtained two series of parthenogenetic lines, each one after the first obtained by inbreeding from the line preceding. In one of these series, numbered I. in the table, the numbers of families in the six lines were 12, 4, 5, 17, 5, and 9, respectively; in the other series (II.) the numbers of families in successive lines were 12, 10, 8, 11, 21, and 17, respectively.

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[1] Contributions from the Zoölogical Laboratory of the University of Michigan No. 139.

I shall attempt to show, in what follows, that there is a progressive decrease in the vigor of these six lines, from first to last.

TABLE I.

SHOWING DECREASE OF VIGOR, AS MEASURED BY VARIOUS CHARACTERS, IN SIX SUCCESSIVELY INBRED PARTHENOGENETIC LINES OF *Hydatina senta*.

Series.	Character to be Measured.	Number of Parthenogenetic Line.					
		1	2	3	4	5	6
I.	Size of family of parthenogenetic female...	48.4	42.5	46.8	42.5	31.0	22.6
	Size of family of fertilized sexual female...	16.7	12.8	12.8	11.5	6.3	7.3
	Number of eggs laid per day............	11.0	11.4	10.3	10.0	9.2	7.5
	Number of days required to reach maturity	2.27	1.66	2.25	1.93	2.25	2.12
	Proportion of cases in which first daughter did not become parent...............	1/11	1/3	2/4	3/16	0/4	5/8
	Same in percentages..................	14.2		25.0		41.6	
II.	Size of family of parthenogenetic female...	48.4	30.8	41.0	37.0	33.8	24.8
	Size of family of fertilized sexual female...	16.7	13.7	13.5	15.2	10.1	7.6
	Number of eggs laid per day............	11.0	11.6	7.9	7.7	9.6	8.6
	Number of days required to reach maturity	2.27	1.55	2.57	2.20	1.90	2.00
	Proportion of cases in which first daughter did not become parent...............	1/11	4/9	2/7	2/10	8/20	7/16
	Same in percentages..................	25.0		23.5		41.6	

THE MEASURE OF VIGOR.

Six distinct means of measuring the vigor of the several parthenogenetic lines are available. They are as follows:

1. Size of family of parthenogenetic females. With few exceptions every daughter of a female used for breeding was isolated and recorded. The average size of family was computed for each parthenogenetic line separately. Families not completely

recorded, of which there were a few, were not included in this computation.

2. Size of family of fertilized sexual females. It appears that the male does not appreciably affect the number of eggs laid by the female with which he is mated. The size of family is determined almost wholly by the female herself. In determining size of family in this case, I have counted the eggs laid, not the number that hatch, as experiments have led me to conclude that the vigor of the parent is responsible for the number of eggs, but not for their viability.

3. Number of eggs laid per day. The young rotifers were isolated and recorded about the same time each day. To find the average number of eggs per day in a given line, the total number of offspring hatched by that line was divided by the number of days on which they were produced, the days for each family being counted separately. Each family was produced usually in three to five days. In a line comprising nine generations, therefore, the number of days as used in this computation would be 27 to 45, regardless of the fact that several families were producing young at the same time, and that the experiment covered only about 20 days. The first day and the last day on which a given female produced daughters were counted as half days, which they must have been on the average, since the offspring recorded as of one such day must have been in some cases the output of a few hours, in other cases of practically a whole day. It is assumed that the eggs hatched in fairly uniform time, as observations have shown that they do.

4. Number of days required to reach maturity. By this is meant the interval between the laying of an egg and the time when the female that hatches from it begins to lay eggs. This time varies considerably in different lines even when reared under identical conditions. It has been found in some cases that in one line that has passed through over ninety generations parthenogenetically, about three days are required to reach maturity, while a young and vigorous line reared at the same time required less than two days. In the present experiments the time required to reach maturity is an average of the parents of all the generations in a given line. It is the interval between the hatching of the first daughter of the first generation and the hatching of

the first daughter of the last generation, divided by one less than the number of generations.

5. Proportion of cases in which the first daughter did not become the parent of the next generation. In all my breeding experiments, whenever a new family was started, the first two daughters were set aside for further breeding, though only one of them was ordinarily used. If the first daughter was apparently healthy and vigorous, she was invariably used. If the second daughter was distinctly more vigorous than the first, the second became the parent of the next generation. Sometimes it was deemed advisable to discard both and use the third, fourth, or fifth daughter. Thus, in a vigorous line, the first daughter should usually be healthy enough for breeding. As vigor decreased there should be an increasing proportion of cases in which the first daughter was replaced by a later member of the family. I was unconscious of any selection that would have favored other than the first individual in the later lines of each series, for the idea of measuring vigor by this method did not occur to me until the experiments were all finished and the data were being compiled.

6. Difficulty of rearing. As the primary purpose of the experiments was not to test vigor, but to obtain a large amount of data regarding egg characters, every effort was made to keep the conditions of nutrition, chemical composition of the medium, etc., at the optimum. To this end the food cultures were changed as frequently as seemed advantageous. If the rotifers became less vigorous, they would be more sensitive to changes in the food cultures, and it would be necessary to renew the latter more frequently.

RESULTS OF THE EXPERIMENTS.

The first five of these measures of vigor can be expressed in figures. The sixth, though not thus expressible because records were not preserved, is not less valuable. Table I. gives the data under the first five headings.

The table shows that, notwithstanding fluctuations, there is an evident decrease in the size of family, of both parthenogenetic and sexual females, from the first line of each series to the last line.

Notwithstanding fluctuations that are usually small, but occasionally large, the number of eggs laid per day is noticeably less in the later lines than in the earlier ones.

The number of days required to reach maturity remained practically unchanged throughout each series, though the temperature was higher in the later experiments than in the earlier. The first line was reared in November, the last line in June. The higher temperature in May and June should have reduced the time required to reach maturity in those months. In June of the preceding year, two lines which were the ancestors of those given in Table I., but had not been inbred, showed an average time of 1.42 days and 1.56 days, respectively, to reach maturity. The fact that the rate of growth remained practically unchanged in the inbred lines, notwithstanding the increase of temperature in the later experiments, favors the conclusion that the later lines were less vigorous.

The proportion of cases in which the first daughter of a family was not vigorous enough to become the parent of the next generation shows so much fluctuation in the six lines separately that I have combined them two by two. Although the irregularities are not thereby completely removed, it is plain that the later lines include a larger proportion of families bred from other than the first daughter than do the earlier lines.

In regard to the sixth measure of vigor, the difficulty of rearing, figures are not available because records were not preserved. It was evident at the time of the experiments, however, that the difficulty of keeping suitable food cultures gradually increased as the inbreeding proceeded Whereas each culture was satisfactory for three or four days in the first experiments. they usually lasted less than two days at the end. This was not due to chemical changes hastened by higher temperatures in the later cultures, for cool periods in the last experiments, when the temperature was lower than the room temperature maintained in the first experiments, did not make the cultures last as long as in the earlier lines. Furthermore, that nothing was wrong with the food cultures themselves was shown by rearing rotifers from an entirely different source, presumably not often inbred, and obtaining from them healthy and vigorous families. The rotifers of the latter experiments must have been more sensitive to adverse conditions.

With all these measures pointing in the same direction, the evidence of decrease of vigor with successive inbreeding seems conclusive.

Other Evidence Relating to Vigor in Inbreeding.

In one of my earlier experiments (Shull, 1911), two parthenogenetic lines of *Hydatina* were crossed and a new line started from one of the F_1 fertilized eggs. The F_1 line was more vigorous, as measured by size of family and rate of growth, than was either parent line. This increased vigor in F_1, which Whitney (1912) has since shown to be general in *Hydatina*, is without doubt the same phenomenon as the decrease of vigor with inbreeding. Later experiments of my own (Shull, 1912), which involved inbreeding twice in succession, though affording some evidence of an attendant decrease of vigor, were in part contradictory. In the light of the present evidence, these contradictions appear to be merely the fluctuations, such as are found in Table I., and all that was needed to clear them up was further inbreeding. So far as *Hydatina* is concerned, there is thus entire agreement in the results of different experiments and different investigators.

In other animals and in plants there has been accumulated a large amount of information leading to the same conclusion, though not without exception, that inbreeding is attended by deterioration. On the animal side it has long been a commonplace among practical breeders that inbreeding, at least in many cases, is followed by a weakening of stock. Results of scientific value have been reported in recent years. Castle (1906) found that inbreeding the fruit-fly *Drosophila* probably reduces productiveness slightly (though this reduction could be prevented by selection). Moenkhaus (1911) inbred the same fly (*Drosophila*) and found the operation attended by a considerable increase of sterility (failure of the eggs to hatch). He was not inclined, however, to class sterility as a loss of vigor, since other attributes of vigor were not perceptibly diminished. More decidedly favoring the view that inbreeding reduces vigor is the older work of von Guaita (1898) on the mouse and Ritzema Bos (1894) on rats.

The great stature of plant hybrids was noted by Kölreuter (1763); numerous examples were cited by Gärtner (1849); and experiments with many plants were recorded by Darwin (1876). The value of crossing was known to Beal (1876) who made recommendations in regard to the rearing of corn and other plants of commercial value. More recently there have been a number of discussions, accompanying new evidence from plants. Maize has shown in the hands of G. H. Shull (1908), East (1908), and Collins (1910) that inbreeding is accompanied by deterioration, and that crossing between distinct lines brings about an increase of vigor in F_1. East and Hayes (1912) obtain similar results in some crosses of tobacco, though not in all, and Wellington (1912) finds that the yield of tomatoes is increased by hybridization.

Further citation of such evidence would be superfluous, as rather full bibliographies have been given in recent papers, notably that of East and Hayes (1912).

Mendelian Explanations of Vigor.

To explain the cases in which inbreeding is accompanied by deterioration, several theories have been advanced in recent years. Some have suggested that inbreeding greatly increases the chance of producing pure recessive combinations; it is necessary to assume also that these recessive characters are bad. But others have pointed out that there is equal chance that individuals homozygous for good qualities may be produced.

Two other Mendelian explanations have been offered. One was proposed by G. H. Shull (1908) to explain the greater vigor of F_1 plants of common maize. He found that successive self-fertilization in corn reduced vigor rather rapidly at first and more slowly afterwards, while crossing two unrelated lines resulted in much more vigorous plants in F_1. He attributed the vigor of F_1 to its "hybridity," and the gradual reduction of vigor with self-fertilization to the gradual establishment of the homozygous condition. Whether there is supposed to be a special set of genes for vigor, or whether heterozygosis in ordinary body characters is held responsible for vigor, Shull does not state. Presumably the genes are not all equipotent, so that heterozygosis in one character may contribute more to vigor than heterozygosis in another character. A similar view is advocated by East and Hayes (1912), but these authors specify that heterozygosis is responsible for only part of the vigor of an individual. The remainder they speak of as "inherent natural vigor" and leave it unexplained.

The other Mendelian explanation is that of Bruce[1] (1910). According to Bruce's view, there is an indefinite number of genes concerned with each element of vigor, for example, size. Each element of vigor depends on the number of genes present, but dominance is complete, or nearly complete, so that $MmNn$ contributes as much, or nearly as much, to vigor as does $MMNN$. All these genes are held to be equipotent so that $MmNn$ contributes twice as much to vigor as MM, and just as much as $XxYy$. Vigor is therefore proportional, on this view, to the number of different kinds of gene present, whether in homozygous or heterozygous condition. Essentially the same explanation—the bringing together of dominant characters in the zygote, some of which existed in one parent, others in the other parent—was later offered by Keeble and Pellew (1910) to explain greater stature in F_1 hybrids of certain peas. This would, of course, produce heterozygosis in these characters, but it was the presence of the genes, not their heterozygous condition, to which the authors appealed as an explanation.

As between these last two Mendelian views, the evidence does not now decide; but if either one is correct, the other can, with sufficient work, be proven incorrect.

On Shull's view, according to which vigor depends on the number of genes for which the individual is heterozygous, although a single inbreeding of a heterozygous line or a single selfing of a heterozygous individual might, in a few cases, produce offspring heterozygous in just as many genes, and therefore just as vigorous, as its parents; yet successive inbreeding or selfing must, by the laws of chance, eventually result in pure homozygous individuals (homozygous for presence or absence it matters not which). Thus in every pure line (which by definition is homozygous) the minimum of vigor has been reached, and that minimum must be the same[1] for every pure line. Inbreeding must, on this view, always eventually reduce vigor if there is random segregation and recombination.

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[1] This statement of Professor Bruce's view is taken in part from his paper, in part from correspondence with the author.

On Bruce's view, according to which vigor depends on the number of different kinds of gene present, there ought to be some cases in which the inbreeding of a heterozygous line or the selfing of a heterozygous individual would result in F_1, or F_2, or F_3, etc., having as many present genes as the parent. Thus a parent having the constitution *AaBbCcDd* might, on selfing for one or more generations, have a few progeny with the formula *AABBCCDD*. Such individuals should be as vigorous as the original parent, or, if dominance is not complete, even more vigorous; and their progeny, produced by self-fertilization, should never show decrease of vigor. Any individuals that came to have the constitution *AABBCCdd* would be a little less vigorous. Those having the formulas *AABBccdd* and *AAbbccdd* would be still less vigorous, and the constitution *aabbccdd* would represent the minimum of vigor. Thus, if four genes were concerned with vigor, it should be possible to isolate four pure lines, each with its own degree of vigor, which would not thereafter decrease. Each line reaches a minimum of vigor, but that minimum is not the same for different lines.

Either of these two views, as stated, fits the evidence so far obtained from cases in which inbreeding reduces vigor, though evidence could be obtained, at the expense of sufficient labor, which would not fit both. East and Hayes's addition to the heterozygosis view, which would probably be assented to by Shull, namely, the postulation of an "inherent vigor" not dependent on heterozygosis, would make it possible to produce pure lines having different degrees of irreducible vigor. But in no case could a pure line, derived by successive inbreeding from an F_1 that was more vigorous than its parents, be as vigorous, on the view of Shull, or of East and Hayes, as the original F_1; this would be possible, as explained above, on the view of Bruce. If many genes were concerned with vigor, testing the correctness of the two hypotheses on the basis of this distinction would probably require a prohibitive amount of labor.

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[1] Note, however, the effect of East and Hayes's addition to this theory, discussed below.

The view that vigor depends upon the heterozygosis of the individual seems to me inherently more probable than that it is due to the presence of certain dominant genes. The former view admits of a plausible foundation in cell physiology, and the essence of it may be extended to cases of decrease of vigor in which there is no change in the genotypic constitution, and which are therefore without the pale of either theory.

Loss of Vigor Not Accounted for by the Mendelian Explanations.

It has been shown in a former paper (Shull, 1912) that parthenogenetic lines of *Hydatina* may, and usually do, become less vigorous as parthenogenesis proceeds. This conclusion has been confirmed by Whitney (1912). The same phenomenon, though disputed by Woltereck (1911), has been reported in *Cladocera* by Papanicolau (1910). Some workers have found that clones of *Paramecium* decrease in vigor with long continued fission, though Woodruff (1911) has shown that this phenomenon is not universal. In none of these cases, so far as known, is there any change in the genotypic constitution throughout the line; hence a change from heterozygosis to homozygosis is not responsible for the decrease of vigor. The loss of vigor usually spoken of as senescence likewise occurs without, so far as known, any change in zygotic constitution.

The physiological explanation which I am about to offer includes the view that heterozygosis determines vigor, and covers also the cases of parthenogenetic lines and clones just mentioned, and perhaps also of senescence.

A Physiological Explanation of Vigor.

Vigor may be thought of as dependent on the rate of metabolism. Lillie (1912), in his studies of fertilization, concludes that the increased metabolism that accompanies the development of the egg is due to an accelerated interaction between nucleus and cytoplasm. The introduction of new nuclear elements into the cytoplasm of the egg, which occurs in cross fertilization, may be supposed to disturb the equilibrium, create a greater reaction between nucleus and cytoplasm, thereby increasing metabolism, and hence vigor. On this view, it is not the fact that the constitution of the F_1 individuals is *Mm* that makes them vigorous, but the interaction of a nucleus[1] of constitution *Mm* with a mass of cytoplasm accustomed, so to speak, to a nucleus of constitution *MM* or *mm*. If it were possible to remove from an egg its own nucleus, and substitute for it a nucleus slightly different, but not so different as to be "incompatible," with a diploid set of chromosomes, and have it develop normally, it should, on my view, produce an individual more vigorous than its parent, even if the introduced nucleus were completely homozygous. On this view, a line that has become homozygous need not have reached its minimum of vigor, as it must on both of the other views discussed.

In animals that reproduce by parthenogenesis or fission, the long continued interaction between cytoplasm and nuclei that suffer no change of genotypic constitution, may bring about an approach to equilibrium, thereby decreasing metabolism, and hence vigor.

In like manner, continued production of somatic cells without change of genotypic constitution in the nucleus may cause an approach to equilibrium resulting in senescence in the metazoan individual. The cases in which a high standard of vigor is maintained notwithstanding inbreeding, as in wheat and tobacco, or in the absence of genotypic change, as in Woodruff's paramecia, are not so easily explained. They may be due to any one of several causes. If metabolism be maintained by a reversible reaction between nucleus and cytoplasm, vigor could be sustained indefinitely. Or the interaction may be kept up by changes in the cytoplasm, changes due to variable nutrition or other external agents. These are mere suggestions.

East and Hayes have suggested a physiological foundation for the heterozygosis view. They hold that increased vigor in hybrids is due to more rapid cell division, and that the stimulus to this more rapid division is given by the presence of genes in the heterozygous condition. To me it seems that the stimulus is due, not to any effect that the two parental contributions to the nucleus may have directly upon one another, but to the effect of a changed nucleus and a (relatively) unaltered cytoplasm upon each other.

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[1] It is assumed without argument that the representatives of body characters reside in the chromosomes.

Perhaps this suggestion must remain in part mere speculation; but the science of cell physiology is still young, and much may be discovered that will make the proposed view either probable or improbable.

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THE OTOCYST OF THE PINNIDÆ.

B. H. GRAVE,
University of Wyoming.

The conclusions reached in this paper were drawn from a study of the otocyst in three species of lamellibranch belonging to the family Pinnidæ.

I became interested in the subject some time ago, when I found what appeared to be an abnormal otocyst in *Atrina rigida*.[1] In the paper referred to, it was intimated that the otocyst of this lamellibranch showed signs of degeneration. Further study of this organ in the same and related species has convinced me that the otocyst of the Pinnidæ is functionless.

The following description was written after examining something over sixty specimens by the method of serial sections.

Unlike the homologous structure of other lamellibranchs, this sense organ is situated in the tip of the foot at a very considerable distance from the pedal ganglion. In general, it resembles the ordinary lamellibranch otocyst, but differs in being exceedingly large and compound. (See Figs. 1, 2 and 3.)

Instead of being a simple capsule, it ordinarily consists of several lobes, each containing an otolith. (See Fig. 3.) The lobes are quite variable in size, but as a rule they are remarkably large. One by measurement is 780 microns in diameter, while the enclosed otolith measures 520 microns. It will be noted that an object of this size can readily be seen with the unaided eye. In fact the otocyst in question stands out quite prominently.

By reference to the figures it will be noted that the larger number of the lobes of these otocysts lie in contact with each other. This together with the fact that the cavities of two adjoining lobes are frequently found to be in open communication, indicates that they were formed from an original otocyst by budding. It seems, therefore, that budding accounts for the compound nature of these otocysts.

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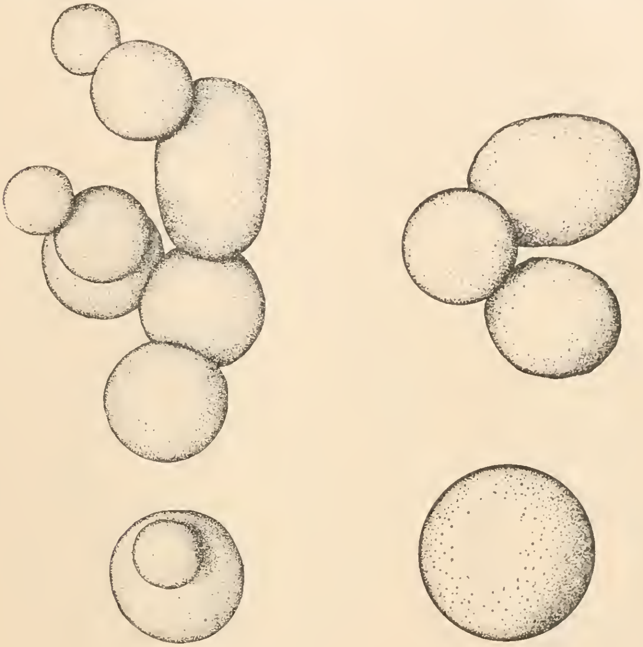
[1] B. H. Grave, "Anatomy and Physiology of *Atrina rigida*," *Bulletin of the Bureau of Fisheries*, Vol. XXIX., 1909.

Be this as it may, the lobes are not all joined in a single mass. In fact, all of the cases figured show two isolated groups. The series of sections from which Fig. 1 was reconstructed shows that this particular otocyst was formed by two separate invaginations from the ectoderm. The tubes indicating their ectodermal nature still persist, as illustrated in Fig. 4. The same ectodermal origion of the otocyst of another specimen, not here figured, is demonstrated by similar ectodermal tubes.



1 2

FIGS. 1 and 2 are reconstructions of the two otocysts of a single specimen. Note that each is composed of two separate groups of capsules. Note also that the appearance is such as to suggest its origin through a process of budding.

It is generally conceded that the otocysts of all mollusks are ectodermal in origin. but it is unusual for their connection with the ectoderm to remain intact in the adult. Such a connection has not. up to this time been observed except in the primitive unspecialized Protobranchia.[1, 2]

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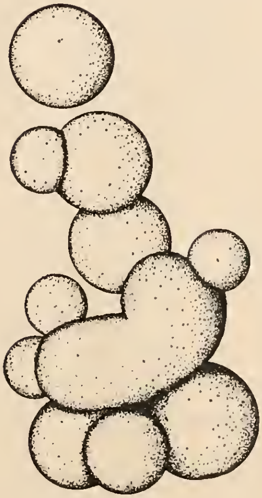
[1] Drew, G. A., "Life History of *Nucula delphinodonta*," *Quarterly Jour. Micr. Sci.*, Vol. 44, Part 3, new series.

[2] Lankester, E. Ray, "A Treatise on Zoölogy," Part V., page 18.

It appears that most of the individuals of the Pinnidæ lack the otocyst altogether. There is not a trace of such an organ to be found in nine tenths of the specimens. In all, I have found only six with otocysts and in these they are highly variable in size and shape.

Of five specimens of *Pinna nobilis* from the Mediterranean, not one had an otocyst, and an equal number of specimens of the small red Pinna from Jamaica[1] were examined with the same negative result.



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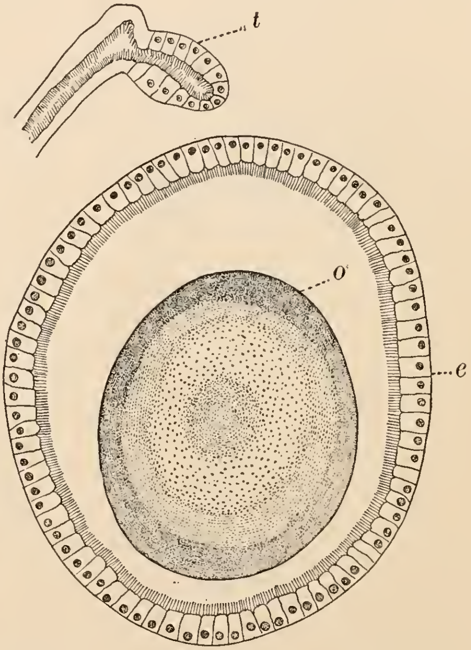

4

Fig. 3. Reconstruction of a particularly compound otocyst.

Fig. 4. Drawing of a section of a single capsule of an otocyst, outlined with a camera lucida. *o*, otolith; *e*, ciliated epithelium; *t*, ectodermal tube which gave rise to the otocyst.

On anatomical grounds, therefore, one is almost justified in concluding that the otocyst of the Pinnidæ is functionless.

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[1] I am indebted to Professor E. A. Andrews for specimens of this Jamaican species.

PHYSIOLOGY OF THE OTOCYST.

While at Beaufort, North Carolina,[1] during the summer of 1911, the writer made a study of the function of the otocyst of *Atrina rigidia*, but the experiments gave only negative results.

A considerable number of specimens were brought into the laboratory and kept under observation to learn their individual behavior. They seemed to suffer no inconvenience after the removal of the tip of the foot, which supposedly contained the otocyst. The normal activities were continued after the operation as before. The following conclusions seem justifiable:

First. A large per cent. of Pinnas have no otocyst. When one is present, it is abnormally large and curiously pathological in appearance.

Second. Anatomical and physiological evidence seem to indicate that the otocyst of the Pinnidæ is undergoing degeneration, and is at present of no functional value.

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[1] I wish to acknowledge my indebtedness to Hon. George M. Bowers for the use of a table at the fisheries laboratory during my stay at Beaufort.

ON THE BREEDING HABITS OF BUTLER'S GARTER-SNAKE.

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Relatively few observations have been made on the breeding habits of snakes, at least few have been published, and there has appeared in print considerable misinformation on the subject, due principally to wrong identification of species. There is especially a deplorable lack of careful studies of the life-histories of the different forms.[1] Concerning the North American forms, we know that some are ovo-viviparous and others oviparous, that copulation probably takes place ordinarily in the spring, although in one species it has been said to occur also in the fall,[2] that the young appear in the late summer, and there is some evidence that there is a gregarious tendency in the breeding season that may lead to the formation of "piles" of snakes.[3] But of the exact time of copulation, the courtship reactions, the significance and commonness of the "snake piles," the length of the gestation period in the different forms, and kindred subjects only the most meager data have been gathered.

During the present year the writer has been able to get a pair of Butler's garter-snake (*Thamnophis butleri* Cope) to breed in captivity and has carried the female over the period of gestation. On April 9, which was about the first day in spring when the snakes were at all active in this region, a male and female of this species were found together near Ann Arbor. These failed to copulate in captivity, although the male courted the female assiduously for several days. On April 10, seven specimens were collected, and a lot consisting of five males and a large female placed together in a cage. The female was courted by the males for five days before she appeared to be ready for copulation.

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[1] An excellent summary of the data on the breeding habits of certain North American snakes is given by O. P. Hay, *Proc. U. S. Nat. Mus.*, XV., pp. 385–398.

[2] Coues and Yarrow, *Bull. Geol. and Geog. Surv. Terr.*, IV., 278.

[3] For a résumé of the literature on this subject see Ruthven, A. G., *Bull. U. S. Nat. Mus.*, 61, pp. 13–14.

The method of courtship is exactly as described[1] for *T. sirtalis*. The male or males lie on or closely along side of the female, keep up at intervals a spasmodic movement of the abdomen, and endeavor to maintain a loop of the tail over that of the female and to insert the posterior part of the body, ventral side up, under hers.

Early on the morning of April 15 five males were at the same time endeavoring to copulate with the female, showing at once that the sexual impulse was at its height and confirming the conclusion that the so-called snake piles are due to this impulse. The exact moment of copulation was not observed but it was within a few minutes of 12 noon. When one of the males had succeeded in inserting one of its hemipeni—the right—in the cloaca of the female the other males at once crawled away.

The pair remained *in coitu* for two hours and fifteen minutes. During that time the male endeavored to maintain a position along the back or close to the side of the female and when in this position kept up the abdominal movements, but the female moved rather constantly about, dragging the male often at full length behind her. Occasionally she rolled rapidly over and over as many as ten times, turning the body of the male at the same time,[2] but this did not break the connection, confirming Cope's[3] statement that the hemipenis cannot be withdrawn except by invagination.

When the act of copulation was completed the male was removed and the female carefully cared for. She ate freely, was fed as much as she would eat, and was little disturbed. Under this treatment she remained in the best of health and on September 6, about 10 A.M., gave birth to thirteen young. This makes the period of gestation almost exactly 144 days.

It should be remarked that either the length of the period of gestation varies, or the breeding season is of some length and depends upon whether the spring is early or late, for the writer has recorded the birth of a brood as early as August 7,[1] and on August 25, 1912, a young specimen collected at Ann Arbor and at the most but a few days old was received by the museum. It is very probable that the length of the gestation period is rather exact for the species, that the snakes breed approximately as soon as the weather is warm enough to permit them to become active, and that the breeding season is about a month long. In southern Michigan the snakes rarely appear as early as the second week in March and usually before the first week in April, so that in this region the general breeding season probably extends from the latter part of March over most of April.

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[1] Ruthven, A. G., *loc. cit.*, p. 178.

[2] More or less of this restlessness of the female may have been due to her being in captivity.

[3] Cope, E. D., Rept. U. S. Nat. Mus., 1898 (1900), p. 701.

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[1] Ruthven, A. G., *loc. cit.*, pp. 90–91.

THE INITIATION OF DEVELOPMENT IN CHÆTOPTERUS.

HARRIETT M. ALLYN.

Page

I. Introduction 22

II. First changes in development 27

III. Study of artificial parthenogenesis in *Chætopterus* by means of single agents 30
- *A*. The action of salt solutions 30
  - 1. Potassium chloride 30
  - 2. Sodium chloride 35
- *B*. Acids 36
- *C*. Alkali 37
- *D*. Alcohol 37
- *E*. Potassium cyanide 38
- *F*. De-aerated sea-water 38
- *G*. Excess of oxygen in the sea-water 38
- *H*. Temperature changes 39
  - 1. Cold 39
  - 2. Heat 39

IV. Combination of two agents 42
- *A*. Artificial parthenogenesis supplemented by fertilization 42
  - 1. Potassium chloride and sperm 42
  - 2. Cold and sperm 46
  - 3. Excess of oxygen in the sea-water and sperm 47
- *B*. Combination of two physico-chemical agents 48
  - 1. Double potassium chloride 48
  - 2. Oxygen and potassium chloride 48
  - 3. Heat and potassium chloride 49
  - 4. Cold and potassium chloride 50
  - 5. De-aerated sea-water and potassium chloride 51
  - 6. Potassium chloride and sea-water de-oxygenated by a stream of hydrogen 51
  - 7. Potassium cyanide and potassium chloride 51
  - 8. Sodium chloride and sodium hydroxide 52

V. Discussion 52
  - 1. Problem of the first changes 52
  - 2. Problem of maturation 54
  - 3. Problem of differentiation 58
  - 4. Problem of cleavage 61
  - 5. Artificial parthenogenesis supplemented by fertilization 63
  - 6. Combination of two physico-chemical agents 64
  - 7. Summary of discussion 65

I. Introduction.

The object of the experimentation described in this paper was a partial analysis of the initiation of development in the egg of the marine annelid, *Chætopterus pergamentaceus.* The experiments were made and material collected at the Marine Biological Laboratory in Wood's Hole during the summers of 1909, '10 and '11.

Two methods of attacking the problem were employed,—the first, that of inducing artificial parthenogenesis,—the second, that of combining fertilization and artificial parthenogenesis. No investigator has yet succeeded in inducing normal development by parthenogenesis in *Chætopterus*, although varying degrees of approximation to this result have been attained. A variety of methods, which have proved more or less effective in other forms, have therefore been employed, in the hope that such experiments might throw light on certain of the factors concerned in the initiation of development in this particular form.

A. Normal Development.—The egg differs from many eggs in having a membrane present before fertilization. About ten minutes after the entrance of the spermatozoan the first polar body is extruded, and ten minutes later the second. The first cleavage takes place forty minutes after fertilization, and is preceded by the formation of a large polar lobe, the contents of which passes into one of the two daughter cells. The first cleavage is thus unequal. The history of the cleavage is essentially like that of other annelids. The blastula becomes ciliated, and swims at six or seven hours. The larval form is a trochophore, uniformly ciliated, with a long apical tuft of cilia at the anterior end. Among any set of normal, fertilized eggs, there are very likely to be found varying numbers of unsegmented, ciliated, swimming larvæ. Cleavage is thus seen to be unnecessary to a ? certain amount of differentiation and development. This was shown experimentally by F. R. Lillie (1902). These larvæ do not develop the typical form nor the apical tuft of cilia.

B. Artificial Parthenogenesis.—In artificial parthenogenesis the development follows much the same course as in fertilization, but the experiments enable us to resolve the development into a separable series of steps. The first step is the formation of the metaphase of the first maturation spindle, the second the extrusion of the first polar body, the third the completion of maturation, the fourth is cleavage (the second, third and fourth may be omitted), the fifth shows varying degrees of differentiation up to the formation of swimming larvæ of greater or less activity. A graded series of stimuli may be determined in which the development may be made to stop at almost any step in the process.

The chief difficulty in the artificial parthenogenesis has been to induce cleavage. Formerly all successful efforts to induce parthenogenesis in *Chætopterus* resulted in the production of unsegmented larvæ only.[1] One of my principal objects has been to discover, if possible, something of the conditions determining cleavage. I have found many agents that will induce the development of swimming larvæ without cleavage, but only two thus far which led to the production of segmented larvæ,—(1) subjecting the eggs to the action of heat, and (2) the use of oxygen-saturated sea-water after a short exposure of the eggs to a potassium chloride solution. The latter gave segmented larvæ in so few cases that I consider the results as somewhat uncertain. Of the heat results there is no doubt. The fact that so many fertilized eggs develop without cleavage suggests that any abnormal condition is likely to have as a result the suppression of cleavage,—that the *balance* of developmental phenomena is a delicate one. The variety of agents that will induce development of some sort indicates that the egg is in a rather easily disturbed equilibrium,—a very *labile* condition.

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[1] Since sending this paper to the BULLETIN I have read the account by Professor Jacques Loeb and Dr. Hardolph Wasteneys, "Fertilization of the Eggs of Various Invertebrates by Ox-Serum," published in *Science*, Vol. 36, No. 921, 1912. In this paper they announce the production of cleavage in unfertilized eggs of *Chætopterus* by the following method. "We induced segmentation in the eggs of *Chætopterus* by putting them for from 1½ to 2½ minutes in a mixture of 25 c.c. $\frac{3}{8}\,M$ strontium chloride + 25 c.c. $M/2$ NaCl + CaCl + KCl, then for ten minutes into ox-serum diluted with its own volume of the above mentioned solution and then by putting them for thirty minutes into hypertonic sea-water." The parthenogenetic agent is considered to be the lysin in the ox-serum, and the authors' conclusion is for *Chætopterus* as for the sea-urchin, that the first phase of fertilization consists in a superficial cytolysis.

TABLE I.

AGENTS INDUCING THE FORMATION OF SWIMMING LARVÆ UNSEGMENTED EXCEPT IN THE CASE OF HEAT, OR OF KCL FOLLOWED BY OXYGEN EXCESS IN THE SEA-WATER.

- Potassium chloride, 2½ *M* in sea-water.
  - 0.5 per cent.—10½ minutes to 5 hours and 10 minutes.
  - 1.0 per cent.—3 minutes to 5 hours and 10 minutes.
  - 2.0 per cent.—21 minutes to 3 hours and 10 minutes.
  - 3.0 per cent.—45 and 60 minutes. (Shorter exposures not made.)
  - 3.5 per cent.—1 minute to 3 hours.
  - 4.0 per cent.—45 to 60 minutes. (Other exposures not made.)
  - 5.0 per cent.—1 minute to 3 hours and 10 minutes.
  - 6.0 per cent.—45 and 60 minutes. (Other exposures not made.)
  - 7.5 per cent.—5 minutes to 4 hours.
  - 10.0 per cent.—5 minutes to 3 hours and 58 minutes.
  - 15.0 per cent.—1 minute to 31 minutes.
- *n*/10 acetic acid in sea-water.
  - 1.0 per cent.—10½ minutes.
  - 5.0 per cent.—5 minutes.
- Low temperature.
  - 9.5° C.—1 hour or 2 hours and 17 minutes.
  - 12.5° C.—2 hours and 17 minutes.
- Oxygen-saturated sea-water.
  - 1 hour to 2 hours.
- De-aerated sea-water (after 2 per cent. 2½ *M* KCl 3 minutes)—over night.
- De-aerated sea-water (with 2 per cent. 2½ *M* KCl)—1 hour and 30 minutes.
- Alcohol in sea-water.
  - 5 per cent. 40 minutes.
- High temperature.
  - 33°–34° C.—25 minutes to 1 hour and 30 minutes.
  - 32.5°–34.5°—40 minutes to 1 hour and 30 minutes.
  - 30°–30.5°—40 minutes.
  - 33.5°–34.5°—25 minutes to 40 minutes.
- Sodium chloride, 2½ *M* in sea-water.
  - 13.7 per cent.—5 minutes and 1 hour and 10 minutes.
- 2½ *M* sodium chloride and *n*/10 sodium hydroxide, in sea-water.
  - 8.0 c.c. NaCl + 50 c.c. sea-water + 0.2 c.c. NaOH—20 minutes to 18 hours.
  - 8.0 c.c. NaCl + 50 c.c. sea-water + 0.4 c.c. NaOH—20 minutes to 18 hours.
  - 8.0 c.c. NaCl + 50 c.c. sea-water + 1.0 c.c. NaOH—50 minutes to 18 hours.
- *n*/10 hydrochloric acid in sea-water.
  - 2 per cent. permanently.
- Fatty acid followed by hypertonic sea-water.
- *n*/10 butyric acid 2.8 c.c. + 50 c.c. sea-water—2 minutes; then 200 c.c. sea-water 20 minutes; then 8.0 c.c. 2½ *M* NaCl + 50 c.c. sea-water 30 minutes.
- Potassium chloride followed by hydrogen current through sea-water.
  - 3.5 per cent. 2½ *M* KCl in sea-water—45 or 65 minutes, then hydrogen 3 hours.
- Potassium chloride followed by excess of oxygen in sea-water.
  - 3.5 per cent. 2½ *M* KCl in sea-water until after formation of second polar body, then oxygen saturated sea-water 30 min. to 4 hours.

TABLE II.

AGENTS INDUCING POLAR BODY FORMATION, BUT NO SWIMMING LARVÆ.

2½ *M* potassium chloride in sea-water.
  0.1 per cent.—1 minute to 25 minutes.
  0.5 per cent.—5½ minutes, sometimes 10 or 20 minutes.
  1.0 per cent.—1 minute, sometimes 3–6 minutes.
  2.0 per cent.—1 minute to 10 minutes.
  3.5 per cent.—4 minutes (in some cases).
  5.0 per cent.—4 minutes (in some cases).
  7.5 per cent.—1 minute.
  10.0 per cent.—1 minute.
  15.0 per cent.—5 minutes (in some cases).
n/10 acetic acid in sea-water.
  1.0 per cent.—1 or 5 minutes.
Low temperature.
  9.5° C.—1 hour (in some cases).
  12.5° C.—15 minutes.
De-aerated sea-water.
  10 minutes to 1 hour and 16 minutes.
Oxygen-saturated sea-water.
  16 minutes to 1 hour and 5 minutes.
n/10 potassium cyanide in sea-water.
  20.0 per cent.
Double potassium chloride, in some cases.

TABLE III.

AGENTS INEFFECTIVE IN INDUCING DEVELOPMENT.

Potassium chloride, 2½ *M* in sea-water.
  1.0 per cent.—1 minute (in some cases).
  7.5 per cent.—5 hours and 30 minutes.
  10.0 per cent.—5 hours and 30 minutes.
  15.0 per cent.—1 hour 55 minutes to 5 hours 30 minutes.
Mechanical agitation or nicking.
Low temperature.
  9.5° C.—15 or 30 minutes.
Sperm extract, by boiling.
Alcohol in sea-water.
  0.5 per cent.—3 minutes to 3 hours 15 minutes.
  2.0 per cent.—3 minutes to 3 hours 15 minutes.
  5.0 per cent.—3 minutes to 3 hours 15 minutes (except 40 minutes).
Oxygen-saturated sea-water.
  10 or 20 minutes (in some cases).
High temperature.
  30°–31.3° C.—5 minutes or 1 hour 30 minutes.
  33°–34.2° C.—5 minutes or 20 minutes.
  35°–45° C.—5 minutes to 60 minutes (except perhaps 40°,—1 minute).
Nitric acid.
Sulphuric acid.
Oxalic acid.

Tables I.–III. show a list of the agents employed, with the varying degrees of success. As stated in Table I. swimming larvæ may be induced in unfertilized eggs by the use of potassium chloride, sodium chloride, hydrochloric acid, acetic acid, butyric acid followed by sodium chloride, sodium hydroxide and sodium chloride, alcohol, potassium chloride followed by hydrogen or by oxygen-saturated sea-water, or by de-aerated sea-water, low-temperature, high-temperature, or oxygen-saturated sea-water. Probably there is a large number of other agents also which would bring about the same result. The majority of the above may be made to induce polar body formation only, if shorter time or lower concentration is used (see Table II.). Potassium cyanide, and de-aerated sea-water, both induced polar bodies only, no matter what time or concentration was used. The same agents may prove altogether ineffective in inducing development when used in certain other concentrations and times. Sulphuric, nitric, and oxalic acids, were also found to be ineffective, as well as sperm extract (by boiling), and mechanical agitation and injury (by shaking with ground glass) (Table III.). Many of the experiments were performed merely to obtain a "lead," and if the results did not appear suggestive that line of work was abandoned. If they appeared suggestive the experiment was usually repeated with some changes.

Considerable variation was noted in the behavior of different lots of eggs under similar conditions of experimentation. Just what the conditions are which govern this variation I have not been able to discover. Sometimes the female did not appear to be in good condition, but this was not a universal test by any means. Variations in results under uniform conditions seem to be characteristic of experiments in artificial parthenogenesis.

C. Methods.—The animals were brought to the laboratory in their tubes. On removal from the tubes, the males and females were kept in separate dishes in running sea-water, from twelve hours upward. In some cases the females were washed in tap water for one minute, before using, but this did not seem to be a necessary precaution. On using, the sex segments were cut off, the ovaries removed, and torn to pieces, and the eggs passed through cheese-cloth to free them from body fragments and from some of the mucus, which is very thick.[1]

The eggs were left in sea-water, after removing from the ovaries, from twenty to forty minutes, in order that the first maturation spindle might form before further treatment. Experiments showed that eggs so treated formed a larger percentage of second polar bodies. Such eggs formed the polar bodies more quickly, both in normal fertilization and in experiments. Unfertilized controls were always run, and fertilized controls also if the experiment included the effect of the combination of fertilization and artificial parthenogenesis. Care was taken, of instruments and dishes, to keep all unfertilized eggs free from contamination from sperm.

A considerable amount of material was fixed for cytological study, largely covering the ground of the experiments. The study of the fixed material was used to amplify and check that of the living eggs. The eggs used for this work were killed either in Boveri's picro-acetic; in Flemming's (weak) solution; or in Meves's fluid. They were stained in Heidenhain's iron-hæmatoxylin or in thionin,—and counter-stained in Orange G. The sections were cut four micra thick.

II. The First Changes in Development.

So long as the eggs remain in the ovary the germinal vesicle is intact, but almost immediately upon entrance into sea-water the vesicle breaks down, its contents migrate to the animal pole, and the metaphase of the first maturation spindle forms. At the same time the membrane, which could be seen from the beginning, stands off from the egg at a greater distance, forming a distinct space between the membrane and the egg surface. Here development pauses, and unless further stimulus is given the eggs remain in this condition, and after a number of hours go to pieces.

The question of determining the factors which bring about these first developmental changes is a problem of which I have had

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[1] It is probable that one reason so many of the controls died before the close of the experiment was that more mucus was left in the control dishes than in the ones in which the solutions were changed a greater number of times.

opportunity to make only the most hasty examination. It might well repay further work. The method employed was to open up the ovaries in solutions other than sea-water: The female was rinsed one minute in distilled water, to wash off the sea-water, then dried on filter paper sufficiently to remove external moisture. The sex segments were then cut off and placed in the desired solutions, where the eggs were teased out. The results were as follows:

A. Body Fluid.—When no solution was supplied and the eggs were merely allowed to come out of the ovary into a drop of body fluid (which is plentiful in the sex segments outside the ovary tubes), the germinal vesicle broke down, its contents migrated to the animal pole, the spindle formed, and the membrane assumed its normal relation to the egg. In short the whole process appeared to be normal. Since the body fluid can produce these changes it must be supposed either that the ovarian wall is impermeable to the body fluid, or that an oxygen supply greater than that within the ovary must be furnished in order to induce the changes. To test this last suggestion sex segments were opened up in water which had been de-aerated by boiling.

B. De-aerated Sea-water.—In this case the female had not been dried as formerly, since sea-water was to be used. Eggs taken from the ovary in de-aerated sea-water behaved normally in all respects. It is of course possible that sufficient oxygen was carried in with the eggs to have an effect on the processes, but it scarcely seems probable that much increase in oxygen supply over that present in the ovaries was furnished the eggs in the experiment. Therefore, it would appear that the reason that the eggs do not go through the first stage in the ovaries is that the wall of the ovary is semi-permeable to the body fluid, not allowing a sufficient amount of it to enter to cause the eggs to go through the changes described.

C. Salt Solutions.—In order to determine whether any of the salts in the sea-water were necessary factors in the determination of these first developmental changes, solutions of various salts were used,—$n/2$ sodium chloride, $n/2$ potassium chloride, $n/2$ calcium chloride, and $n/2$ magnesium chloride. The $n/2$ sodium chloride and $n/2$ potassium chloride led to the break-down of the germinal vesicle, the migration of its contents to the animal pole, and the formation of an apparently normal spindle, but not to normal membrane relations. The $n/2$ calcium chloride and $n/2$ magnesium chloride led to the break-down of the germinal vesicle, but the migration of the contents of the vesicle to the animal pole did not follow. In the $n/2$ magnesium chloride the membrane relations were fairly normal,—but not so in the $n/2$ calcium chloride.

Combinations of the salts were made according to the following Van't Hoff formula:

$$\frac{n}{2}\ \mathrm{NaCl}\text{—10 c.c.}$$

$$\frac{n}{2}\ \mathrm{KCl}\text{—0.22 c.c.}$$

$$\frac{n}{2}\ \mathrm{MgCl_2}\text{—1.2 c.c.}$$

$$\frac{n}{2}\ \mathrm{CaCl_2}\text{—0.2 c.c.}$$

When 10 c.c. of $n/2$ NaCl was used with 1.2 c.c. of $n/2$ $MgCl_2$ (no KCl or $CaCl_2$ being present), the results were normal in all particulars. Evidently then, potassium chloride and calcium chloride are unnecessary to the first developmental stage. When, however, 0.22 c.c. of $n/2$ KCl or 0.2 c.c. of $n/2$ $CaCl_2$ were substituted for $n/2$ $MgCl_2$ with $n/2$ NaCl, the spindles formed as normally but the membrane relations were not normal. Magnesium chloride seems to have some definite effect in inducing normal membranes, therefore, and either $n/2$ sodium chloride or $n/2$ potassium chloride may induce normal spindles. Salt solutions are not necessary to induce certain of the developmental changes, however, as is seen by experiments with neutral paraffin oil and with cane sugar.

D. Neutral Paraffin Oil.—When the ovaries were opened in this liquid a certain number of eggs, although not all by any means, appeared to behave normally in that the vesicle contents migrated to the animal pole, and the maturation spindle formed,—but the eggs swelled slightly and the membrane did not stand off from the egg as normally. The result may be complicated by the fact that a small amount of body fluid is always present, and does not mix with the oil. However, where the eggs seemed to be comparatively free from body fluid in the oil, the results were as stated. It is possible that the space between the membrane and the egg may be obliterated by the swelling of the egg.

E. Cane Sugar Solutions.—*n*, 5/8*n*, and *n*/2 solutions of cane sugar were tried. All of the solutions injured the eggs considerably. In the 5/8*n* sugar the membrane swelled away from the egg, leaving a space much larger than normal, while the egg itself appeared to be plasmolyzed. Evidently fluid had been extracted from the egg into the vitelline space by the injurious action of the sugar solution. In a few eggs in all the solutions the germinal vesicle broke down, its contents migrated to the animal pole, and something resembling an abnormal polar spindle was formed.

The experiments are too few to draw from them definitive conclusions. It seems possible, however, that the internal condition of the egg is a sufficient stimulus to cause the first developmental stage, provided that conditions be furnished which are favorable to the reactions involved, that is, that the egg needs no stimulus from without, and that the failure of the germinal vesicle to break down in the ovary is due merely to an inhibition of the processes because of a lack of favorable conditions in the ovary,—that all that is necessary is a release of the process in the egg.

III. Study of Artificial Parthenogenesis in Chætopterus by Means of Single Agents.

A. The Action of Salt Solutions. 1. *Potassium Chloride.*—Mead ('98), Loeb ('01) and F. R. Lillie ('02) used potassium chloride to induce development in *Chætopterus*,—Mead only carrying the eggs through maturation and a few of the earlier developmental changes, Loeb performing a number of experiments to determine the nature of the KCl effect, and Lillie making an extensive study of the course of the development, with living and fixed material. Following their lead I have used potassium chloride as one of my principal agents. The cytological results of potassium chloride treatment are very interesting and appear fairly typical of development without cleavage, induced in any way whatever,—by parthenogenetic agent or by sperm. There is space here for a general account only, but I hope to give a more extended treatment of the subject at another time. The hope was entertained for some time that the right concentration or time of application of potassium chloride might bring about cleavage, but the method has never been successful for that purpose.

In the experiments a certain number of cubic centimeters of 2½ *M* KCl were mixed with a given amount of sea-water. The eggs were placed in this solution after remaining 20–40 minutes in ordinary sea-water (a delay which hastened and aided development),—and later were returned to normal sea-water after a given exposure to the action of the KCl.

A. Experiments to Determine the most Favorable Concentration and Time for Development.—Numerous experiments were performed to determine the best concentration, and time of KCl exposure to bring about the largest percentage of development. Tables IV. and V. are taken from this set of experiments. By comparing the tables it will be noted that there is a considerable difference between the concentration and time inducing the best percentage of maturation and that inducing the greatest production of swimmers. Brief exposures give the best results for maturation, but the greatest number of swimmers arises from longer exposures. The best percentage of maturation,—100 per cent. of eggs completing maturation,—was gained by treatment

TABLE IV.

TO SHOW THE BEST LENGTH OF EXPOSURE TO A GIVEN POTASSIUM CHLORIDE SOLUTION TO INDUCE THE FORMATION OF BOTH POLAR BODIES.

Concentration of 2½ *M* KCl, in Sea-water, Per Cent.	Time of Exposure.	Per Cent. Forming Polar Bodies.
0.1	9.5 minutes	14
0.3	10.5 minutes	29
0.5	10.5 minutes	61
1.0	3–6 minutes	100
2.0	3–5 minutes	81
3.5	1 + minute	100
5.0	1 minute	59
7.5	1 minute	80
10.0	1 minute	41
15.0	1 minute	64.5

TABLE V.

TO SHOW THE BEST LENGTH OF EXPOSURE TO A GIVEN SOLUTION OF POTASSIUM CHLORIDE TO INDUCE THE FORMATION OF SWIMMERS.

Concentration of 2½ *M* KCl, in Sea-water, Per Cent.	Time of Exposure.	Per Cent. Swimmers Formed.
0.1		None
0.5	1½ hours	9
1.0	5 hours	16
2.0	30 minutes	16⅔
3.5	1½ hours	17¼
5.0	3 hours	33
7.5	30 minutes	11
10.0	62 minutes	11.5
15.0	10–20–31 minutes	2.5

TABLE VI.

TO COMPARE EFFECT OF TIME AND CONCENTRATION ON POLAR BODY AND SWIMMER FORMATION.

Concentration of 2½ *M* KCl in Sea-water, Per Cent.	Length of Exposure.	Per Cent. Forming Polar Bodies.		Per Cent. Forming Swimmers.
		Both.	Only First.	
3.5	1 min.	100	0	4
3.5	10 min.	57	28	3
3.5	21 min.	41.6	25	8.5
3.5	90 min.	?	?	17
5.0	1 min.	59	25	8
5.0	10 min.	18	66	6
5.0	20 min.	37	37	10
5.0	3 hr.	?	?	33
7.5	1 min.	80	13	0
7.5	5 min.	41	38	2
7.5	10 min.	50	23	6.5
7.5	20 min.	11	41	4.5
7.5	30 min.	8	39	11
7.5	45 min.	0	8	14
7.5	4 hr.	?	?	1
10	1 min.	41	41	0
10	5 min.	35	45	2
10	10 min.	12	75	1.5
10	20 min.	0	70	4
10	30 min.	4	63	7.5
10	45 min.	0	18	6.8
10	63 min.	?	?	11.5
10	3 hr.	?	?	3
15	1 min.	65	32	1
15	5½ min.	60	33	1
15	20 min.	15	65	2
15	31 min.	0	50	2.5
15	45 min.	0	9	0
15	115 min.	?	?	0

with 1 c.c. of 2½ *M* KCl + 99 c.c. of sea-water for 3-6 minutes, whereas the best percentage of swimmers,—33 per cent.,—was obtained with 5 c.c. of 2½ *M* KCl + 95 c.c. of sea-water for 3 hours. There is a decrease in maturation accompanied by an increase in differentiation as length of exposure to KCl increases. Table VI. will serve to illustrate this point. It was found that in general 3.5 per cent. of the 2½ *M* KCl in sea-water acting for 45 minutes, was most satisfactory for inducing a high percentage both of maturation and of swimmers.

In normally fertilized eggs the requirements for both phenomena, maturation and differentiation, are met at the same time by the entrance of the sperm. Why, then, in the potassium chloride eggs, should the two processes be, as it were, separated, and the optimum conditions for one so far removed from the optimum conditions for the other? It looks decidedly as if there were a distinct set of reactions for the two processes, maturation and differentiation. Whether potassium chloride shall arouse only one, or both of these must be largely a matter of the condition of the individual egg, for in some eggs both processes go on as a result of the potassium chloride treatment, while in the same culture other eggs give only one of the two reactions, or give the other very imperfectly. What those conditions in the egg are, is entirely obscure.

B. Details of Development Induced by Potassium Chloride.—F. R. Lillie has described the course of development of the potassium chloride egg, in his papers of 1902 and 1906. In giving the following account I shall be obliged to repeat many of his observations in order to make the history as clear and complete as possible, but I shall omit details largely. The eggs to be described were from two sets of experiments,—one using 7.5 parts 2½ *M* KCl to 92.5 parts sea-water for one hour (designated set *A*),—the other using 3.5 parts 2½ *M* KCl to 96.5 parts sea-water for 45 minutes (set *B*). The general behavior of the eggs in the two series is very similar, and I shall describe now one, now the other, without distinction.

The Living Egg (*Set B*).—The first polar body forms inside 15 minutes,—the second, when formed at all, inside 30 minutes. The eggs often form a polar lobe and appear to be attempting cleavage. The flow of cytoplasmic material in the egg keeps up in one direction for a time, accompanied by a deepening constriction in the egg, until suddenly the direction of the flow changes, or a new wave is set up, and all appearance of constriction or cleavage is lost. Often the flow is set up in opposite directions at once, and thus the constriction plane is simulated, but one flow suddenly overcomes and obliterates the other. The polar lobes also disappear. The amœboid movements noted in so many eggs are to be seen in *Chætopterus* also, so markedly that at one time successive camera drawings showed that the egg actually moved along the slide for some little distance. Pseudo-cleavage takes place to some extent, that is, cleavage of the cytoplasm without cleavage of the nucleus. There is also indication of amitotic cleavage of nucleus and cytoplasm together. As development proceeds the nucleus increases in size, and a massing of the cytoplasmic materials in different parts of the egg can be seen. At ten or eleven hours the larvæ are beginning to swim,—several hours later than fertilized eggs. The percentage of swimmers at 20–24 hours varies greatly. Usually the larvæ were not kept longer than that, but on one occasion they were kept nearly 48 hours, and at the end of that time I find that my notes record, "Full of very active swimmers, some even having risen in the water, and all much resembling trochophores in general appearance." Many larvæ are able to swim in a comparatively straight course, others spin round and round, others do now one, now the other. There are many fusions and some fragments, any of which may be ciliated. Some are composed of a few pseudo-cells, but many are one-celled and also uninucleate. Vacuoles are noticeable in or near the surface.

Fixed Material.—Eggs from Set *B* were fixed every five minutes for the first two hours and fifteen minutes, and every half hour thereafter to 19 hours 30 minutes, with a few more at five-minute intervals during the period of chromatin distribution. Eggs from Set *A* were fixed every half hour or less.

The history of the development is in general as follows. While the eggs are still in the potassium chloride solution the first maturation division is carried through and the first polar body extruded; the nucleus of the second polar body, if formed, is either extruded or retained within the egg, where it unites with the female pronucleus (see Figs. 1 and 2). After the eggs are transferred to normal sea-water a period of imperfect cleavage follows, which involves only the chromosomes. In the period corresponding to first cleavage, imperfect and rudimentary mitotic figures form and the chromosomes split longitudinally, but do not separate into daughter nuclei. This process is repeated in subsequent divisions and the chromosomes remain in an increasingly large, single mass, of tangled rods. This whole period is characterized by a growth of the chromatin at the expense of the cytoplasm Resting nuclei arise between successive chromosome divisions, as normally, in Set *B*, but not until several divisions have taken place in Set *A*, when a general achromatic period enters. Shortly after this general resting period in Set *A*, and perhaps a little earlier in Set *B*, a change in general behavior follows. The nucleus begins to distribute material to the cytoplasm, sometimes in the form of rods, more often in the form of granules, a certain part of which become indistinguishable in the cytoplasm as chromatin (see Figs. 3 and 4). Part of the chromatin in the cytoplasm seems to be "dissolved"; part forms small granules which are indistinguishable from the small basophile granules of the cytoplasm; part remains as scattered chromidia. The chromatin in the nuclei of some eggs also assumes new forms,—short, stout rods, granular nuclei, karyosomes, and irregular bodies of chromatin. Finally, a large part of the chromatin distributed through the cytoplasm collects in masses in various parts of the egg and forms membranes, thus giving rise to accessory nuclei (Fig. 5). The latter part of development may thus be called a period of chromatin distribution. It is during the latter period that the characteristic cytoplasmic overflow described by Lillie ('06) takes place, followed by the formation of cilia (Figs. 6 and 7). The process of chromatin distribution seems to be definitely related to that of cytoplasmic differentiation.

2. *Sodium Chloride.*—The effects of sodium chloride were similar to those of potassium chloride but not so good,—therefore, the solution was not used to any extent. Eggs in 8 c.c. of 2½ *M* NaCl + 50 c.c. of sea-water for 45 minutes, or for one hour

and 10 minutes showed some unsegmented swimmers, but those left permanently went to pieces (as also in potassium chloride permanently). Something resembling one large polar body was formed in many eggs. Cytological study was not made of these eggs, and therefore I cannot say positively whether it was a polar body or only a small extra-ovate. Morgan ('00) has said that he got no polar body formation with sodium chloride in *Chætopterus*.

B. Acids.—Six different acids were used, three mineral,—*n*/10 hydrochloric, *n*/20 sulphuric, *n*/10 nitric,—and three organic,—*n*/10 acetic, *n*/10 butyric, and *n*/20 oxalic. The results were not particularly satisfactory, and not many experiments were made. It is possible that better results might have been obtained with other concentrations or with different length of application of the acid. The results were as seen in Table VII.

Since polar body formation was not good and the appearance of the eggs was not promising, and since others had tried acids with little effect on *Chætopterus* I did not press the methods further. No normal segmented larvæ developed. As noted in the table, eggs left in 2 c.c. of *n*/10 HCl + 100 c.c. of sea-water permanently, formed a small number of swimmers. Eggs in 1 per cent. of the *n*/10 acetic acid in sea-water, for 1 or 5 minutes, formed polar bodies only, but the same solution for 10½ minutes, or 5 per cent. of the *n*/10 in sea-water, for 5 minutes, induced the formation of a few unsegmented swimmers. Of the ineffective acids oxalic was most promising, and should be tried further.

Butyric acid was used only in connection with sodium chloride and sodium hydroxide, as follows: 2.8 c.c. *n*/10 C_3H_7COOH + 50 c.c. sea-water 2 min., followed by 200 c.c. sea-water 20 min., followed by 8 c.c. of 2½ *M* NaCl + 50 c.c. sea-water 10, 20, 30, 40, 50 or 60 minutes. A very small percentage of partially differentiated eggs, non-swimming, were found next morning and one swimmer was observed in the 30-minute lot, but the hoped-for cleavage was not obtained.

The cytological effects of the acids were similar, in the swimmers, to the potassium chloride effects, but many of the eggs which did not develop far presented a very different appearance, seemingly characteristic of acid treatment. These eggs showed

Table VII.

n/10 Hydrochloric Acid.

Concentration.	Time.	Polar Bodies.	Swimmers.
1 c.c. + 100 c.c. of sea-water	5 min.	None	None
2 c.c. + 100 c.c. of sea-water	5 min.	None	None
2 c.c. + 100 c.c. of sea-water	permanently	None	Few
M/20 Sulphuric Acid.			
5 drops + 100 c.c. sea-water	1 hr. or permanently	None	None
1 c.c. + 99 c.c. sea-water	7 min. or 1 hr. or permanently	None	None
3 c.c. + 97 c.c. sea-water	8 min. or 1 hr. or permanently	None	None
10 c.c. + 90 c.c. sea-water	9 min. or 1 hr. or permanently	None	None
N/10 Nitric Acid.			
6 drops + 100 c.c. sea-water	1 hr.–2 hr.–permanently	None	None
1 c.c. + 99 c.c. sea-water	1 hr.–2 hr.–permenently	None	None
2 c.c. + 98 c.c. sea-water	1 hr.–2 hr.–permenently	None	None
3 c.c. + 97 c.c. sea-water	1 hr.–2 hr.–permanently	None	None
5 c.c. + 95 c.c. sea-water	5 min.–permanently	None	None
10 c.c. + 90 c.c. sea-water	5 min.–permanently	None	None
17 c.c. + 87 c.c. sea-water	5 min.–permanently	None	None
n/10 Acetic Acid.			
a 5 drops + 100 c.c. sea-water	1 hr.–2 hr.–permanently		
b 1 c.c. + 99 c.c. sea-water	1 min.–5 min.–10½ min.–30 min.–1 hr.–2 hr.–permanently.		
c 5 c.c. + 95 c.c. sea-water	1 min.–5 min.–15 min.–31 min.–1 hr.–2 hr.–permanently.		

Results:

a gave no polar bodies or swimmers.

b, 1–5 min. gave polar bodies—no swimmers.

b, 10½ min. gave polar bodies and swimmers.

c, 5 min. gave swimmers.

n/20 Oxalic Acid.

5 drops + 100 c.c. sea-water	1 hr.–2 hr.–permanently	None	None
1 c.c. + 99 c.c. sea-water	1 hr.–2 hr.–permanently	None	None

very little, if any, nuclear growth, but in each egg several asters appeared, without spindles, and with a large, black-staining mass of chromatin (?) at the center of the aster. There was more or less breaking up to form "cells" either nucleate or a-nucleate, but no real, mitotic cleavage.

C. Alkali.—The only alkali used was sodium hydroxide, and as this was used in combination with sodium chloride it will be described under the section on the combined use of two agents.

D. Alcohol.—Very little development followed the use of alcohol in any of the experiments. Concentrations were used of 0.5 per cent., 2.0 per cent., and 5.0 per cent., the solutions being made up with sea-water. The times varied from 3 minutes to 3 hours. One swimmer was found in eggs exposed to 5 c.c. alcohol + 95 c.c. sea-water for forty minutes. The great majority of eggs were undifferentiated. The fact that so few eggs showed any signs of differentiation makes it seem possible that this swimmer was not due to the alcohol, but to some chance sperm, since once or twice I found a swimmer in the control. This was extremely rare.

E. Potassium Cyanide.—Twenty c.c. of $n/10$ potassium cyanide + 80 c.c. of sea-water for forty minutes induced a certain proportion of eggs to form both polar bodies, other eggs only one. Moreover, the polar bodies formed *while the eggs were still in the potassium cyanide solution.* No further development followed. Since KCN is generally considered as an agent which suppresses oxidation, I tried raising the oxygen content of the sea-water after the eggs were removed from the potassium cyanide, hoping that this would induce further development, but the results were negative.

F. De-aerated Sea-water.—The results of the use of de-aerated sea-water were very similar to those induced by potassium cyanide. In both cases we supposedly have reduced oxidation rate. The sea-water was de-aerated by heating to the boiling point in a large flask for five minutes, then cooled in the same flask (rubber stoppered), immersed entirely in water. The eggs were dropped into this bottle. Some air may have re-entered during the course of the experiments. As a result of putting unfertilized eggs into this water a considerable number formed polar bodies, as many as 50 per cent. forming both polar bodies in some experiments, and 15–20 per cent. the first polar body only. The polar bodies formed rather more slowly than normally. No swimmers were formed.

G. Excess of Oxygen in the Sea-water.—Oxygen from a tank (the ordinary commercial oxygen), washed through water, was allowed to fill a wide-mouthed bottle in the bottom of which was about one centimeter depth of sea-water. The bottle was then tightly corked with a rubber stopper, and placed in the ice-chest for two or three days, and shaken a number of times during that time. Before the experiment the bottle was brought into the laboratory. During the course of the experiment a stream of oxygen was run through the bottle. Unfertilized eggs were placed in the bottle of oxygen-saturated sea-water, and removed at various intervals, from 10 minutes to 4 hours, to dishes of ordinary sea-water on the table. The effect of the treatment was to induce the formation of a small percentage of polar bodies and swimmers. With even so short an exposure to excess of oxygen as 16 minutes, 23.7 per cent. of the eggs formed the first polar body and 2.7 per cent. formed both polar bodies,—but with an exposure to oxygen of 55 minutes 16 per cent. formed both polar bodies. A few eggs gave the appearance of one or two cleavages, but no segmented swimmers were found, and less than 1 per cent. of unsegmented swimmers.

H. Temperature Changes. 1. *Cold.*—Eggs placed in dishes of sea-water at room temperature and then removed to compartments of the ice-chest where the temperature was 9.5° C. or 12.5° C. for 15 minutes to 2¼ hours, gave a small percentage of polar body formation and also of unsegmented swimmers. The swimmers were of the ordinary type induced by KCl and described there as apparently typical of unsegmented larvæ.

2. *Heat.*—The use of heat gave by far the best results with *Chætopterus.* I consider the experiments to be described as only preliminary tests, as I did not try the method until the end of the summer, and the supply of eggs was giving out, so that I could not make complete tests by any means. By repeating the experiment a number of times, however, I was able to determine with certainty that heat will not only induce development to unsegmented swimmers in a certain percentage of eggs, but will also bring about the delicate balance of processes concerned in mitotic cleavage of a normal sort. The percentages of swimmers obtained were small, many of the cleavages were abnormal, and nearly all the larvæ became abnormal in time, but they demonstrate clearly that an agent other than the sperm can induce the segmentation of the egg of *Chætopterus.* It may be that some modification of the method. in temperature or time, will lead to the formation of entirely normal larvæ, or perhaps some added agent will be necessary. The only combination which I at-

tempted was with KCl, and that did not result favorably for the production of segmented swimmers.

The method used was to suspend small flasks of sea-water in a water bath whose temperature could be varied, and when the temperature within the flask was at, or a little above, the desired temperature, introduce the eggs. The eggs were thus suddenly subjected to a rise of temperature of several degrees. The apparatus, which was a crude one, did not keep the eggs at an exactly even temperature, and the results will be given in each case as the effect of a temperature within the limits of variation; *e. g.*, the most favorable temperature used was one which varied between 32.5° and 34.5° C. The various temperatures tried ranged from 30° C. to 47° C. From 35° C. up no swimmers were produced, although there was some possible cleavage. 30° C. induced almost no development. The eggs were left in the flask for times varying from 35 seconds to 2 hours and 9 minutes, and then removed to dishes on the table, where the temperature was about 23°–24° C. Other eggs were left in the flasks over night to cool down slowly after the end of the experiment. It is possible that in the very beginning of the experiment the temperature was lowered a little by the introduction of the small amount of water necessarily carried in with the eggs. The optimum length of exposure to the heat was 40 minutes or near it. The very brief exposures were made only for the high temperatures. Less than 25 minutes for the lower temperatures was ineffective in producing segmented swimmers, although shorter exposures than that might show some polar body formation and a small amount of cleavage.

The eggs did not usually extrude the first polar body until removed from the warm water to the cooler, but then went on promptly with one or both maturation divisions. The percentage forming both polar bodies was in general smaller than that forming the first only. In the series of which I have sections the second polar body was not seen to form at all, and only a few eggs formed the first polar body. The series of fixed eggs is not at all complete, as I expected to be able to make other series later. The sections indicate that there is sometimes formation and retention of the nucleus of the first polar body within the egg, for eggs may be seen 24 minutes after being removed from the heat, containing two nuclei and with no polar bodies. These nuclei appear to unite in certain eggs. Other eggs may be seen with one polar body and one nucleus. There were not many eggs in which a count of the chromosomes could be made, but in one such count there appeared to be 18 chromosomes. Evidently the second polar nucleus if formed at all had united with the egg nucleus. I think, however, that the second polar spindle frequently is not formed, but that the chromosomes may go into the resting stage after the first maturation spindle, and emerge from this in the characteristic rod shape of the cleavage divisions, whether one or no polar body has been extruded.

Cleavage proceeds with more or less regularity. Unfortunately the eggs were killed at such time that only one egg was caught with the spindle of first cleavage. It seems to be in early anaphase, and although I cannot count the chromosomes exactly, owing to their twisted rod shape and the direction of the section, their number was approximately 36, which makes it seem that the number normal for cleavage, 18, is probably present in each half of the spindle. Second cleavage stages are wanting also, but eggs fixed two hours from the beginning of the experiment show the four-cell stage with spindles of third cleavage (cf. Fig. 9). In one egg in which I was able to count the chromosomes there appeared to be 18 in each cell. (As before stated, the count is not exact, and only serves to indicate whether the chromosome behavior as to numbers, in these mitoses, is proceeding approximately after the normal order.) At this time many eggs are still seen in the metaphase of the first maturation spindle. This spindle often lies in the center of the egg instead of at the periphery, but is easily distinguished from the cleavage spindle by its smaller size and by the chromosomes, which are in the maturation form. A number of eggs are unsegmented and have multipolar spindles, others are segmented abnormally. Many appear to be developing after the characteristic KCl fashion.

Later sections, between 6 and 7 hours, show many segmented blastulæ (Fig. 10). The blastulæ swim at about this time. Abnormalities are usually to be seen; some larvæ have almost no segmentation cavity, others small extra-ovates. The arrangement of substances in the cells is in general normal. A good many larvæ are abnormally segmented, often one cell having stopped in an early stage of cleavage, and the others having gone on and divided further. The larvæ fixed at 21 hours (Fig. 11) are mostly abnormal, apparently, but one or two were found in the living material which showed even the long apical flagellum.

On the whole the series approaches much more nearly to the normal development than any other series I have so far been able to obtain, chiefly because here, and nowhere else, could I get mitotic cleavage to any extent. The possibility of the development of unfertilized eggs to swimming larvæ having been established, the question of inducing a cleavage process to accompany this development was the next important step. Cleavage obtained, it will now be necessary to vary conditions in such manner that the abnormalities arising in the cleavage with the present method may be avoided. The later development of the larvæ, beyond the trochophore stage, I have not attempted, as my first interest lay in the *initiation* of normal development.

IV. Combination of two Agents.

In order to form a check on the effect of the various agents upon the unfertilized eggs, artificial parthenogenesis was supplemented by fertilization in a number of cases,—sperm being added after the eggs had been treated by a physico-chemical agent. In other cases the effect of supplementing the action of one physico-chemical agent by that of another was tried, and in many instances the results of treatment with two physico-chemical agents were similar to those produced by using one such agent and fertilization.

A. Artificial Parthenogenesis, Supplemented by Fertilization.—Eggs were fertilized after application of an artificially stimulating agent. The parthenogenetic agents used as a preliminary to fertilization were KCl, low temperature, and oxygen-saturated sea-water. In general it may be said that the agents, of whatever sort, which will induce development in unfertilized eggs, are prejudicial to normal development in fertilized eggs.

1. *Potassium Chloride and Sperm.*—A number of experiments were performed to test the effect of fertilizing eggs whose develop-

ment was already initiated by potassium chloride. A set of eggs was placed in 1 c.c. of 2½ *M* KCl + 50 c.c. sea-water. After 1, 3, 15 and 30 minutes eggs were removed from the KCl to sea-water. Each lot was divided, part being fertilized and part remaining in ordinary sea-water with no sperm, to form a KCl control. After 15 and 30 minutes eggs were taken from the 1 and 3 minute KCl controls just described, and fertilized. Thus material was obtained showing the effect of an increasing length of exposure to potassium chloride before fertilization, which could be compared with eggs allowed to stand various lengths of time in ordinary sea-water after a very brief exposure to potassium chloride before fertilization.

TABLE VIII.

	Time in KCl.	Time in Sea-water.		Swimmers, Per Cent.		
				Segmented.	Unsegmented.	Total.
KCl control	1 min.	0	Unfertilized	0	0	0
	1 min.	0	Fertilized	59	17	76
	1 min.	15 min.	Fertilized	20	43	63
	1 min.	30 min.	Fertilized	12.5	45	57.5
KCl control	3 min.	0	Unfertilized	0	10	10
	3 min.	0	Fertilized	38	10	48
	3 min.	15 min.	Fertilized	11	17	28
	3 min.	30 min.	Fertilized	2	10	12
	15 min.	0	Fertilized	8	4	12
KCl control	30 min.	0	Unfertilized	0	16⅔	16⅔
	30 min.	0	Fertilized	5	5	10

As may be seen in Table VIII. the eggs treated for one minute with potassium chloride, and unfertilized, produced no swimmers, whereas those of the same lot fertilized immediately after removal from the potassium chloride showed many swimmers, but of these 17 per cent. were unsegmented. The fertilized control (no KCl) fertilized normally and only two or three unsegmented swimmers were found.

In this particular experiment the record of polar bodies was not taken, but in a similar experiment,—of eggs in 1 c.c. 2½ *M* KCl + 50 c.c. sea-water for one minute,—15 per cent. formed the first polar body, and 11 per cent. formed the second also. As before, no swimmers were formed. When eggs of the same lot were fertilized immediately on removal from the KCl a large percentage extruded both polar bodies,—and both segmented and unsegmented swimmers were produced. The production of a large proportion of the polar bodies and all of the cleavage would appear to be due to the sperm. But that the potassium chloride also had an effect, and opposed to that of the sperm, is seen in the increased percentage of *unsegmented* swimmers in the KCl + sperm material above that in the fertilized control. An exposure to potassium chloride of only one minute thus causes suppression of cleavage in KCl + sperm eggs.

A delay in sea-water before fertilizing after potassium chloride shows a slight decrease in total number of swimmers, and still further suppression of cleavage, making the percentage of unsegmented swimmers much larger, and showing a very marked decrease in segmented swimmers.

When the potassium chloride is applied for a longer time before the eggs are removed to ordinary sea-water the decrease in swimmers is much faster, differentiation to swimming forms being interfered with nearly as much as cleavage, showing a stronger potassium chloride action.

Thus it is seen that the potassium chloride initiates changes which increase with time and which are inimical to normal fertilization. They do not prevent the entrance of the sperm into the egg, but they prevent the normal behavior of the sperm in the egg. Polyspermy is induced, but the suppression of cleavage seems to refer to some other factor also, since in the sections of preserved material no more polyspermy is noted in the case of late fertilization than in early, yet the ill effects of the combination are greater with late fertilization.

Results are similar for 0.5 c.c. 2½ M KCl in 50 c.c. of sea-water, but they were complicated by the fact that the female did not seem to be a good one, for the fertilized control cleaved abnormally and many eggs in it did not cleave at all.

A comparison of the curves made from Table VIII. (Fig. *A*) brings out these facts graphically. *AA* represents the increase in swimmers with increased time in KCl (no sperm). *CC* represents the decrease in swimmers when the eggs are fertilized *at once* on removal from potassium chloride, but with increasing time in potassium chloride. *BD* and *B′D* break this curve up to represent the proportion of segmented and unsegmented swimmers. As is evident *C* represents 76 per cent. swimmers, of which 59 per cent., represented by *B*, were segmented, and 17 per cent., represented by *B'*, were unsegmented. The falling off in percentage of segmented swimmers with increasing time in potassium chloride is much faster than the decrease in percentage of unsegmented. Compare with these curves those showing the effect of a brief exposure to potassium chloride followed by fertilization after varying intervals in sea-water. *CE* represents the decrease in total number of swimmers. It is much less rapid than when the eggs were subjected to potassium chloride during all of the

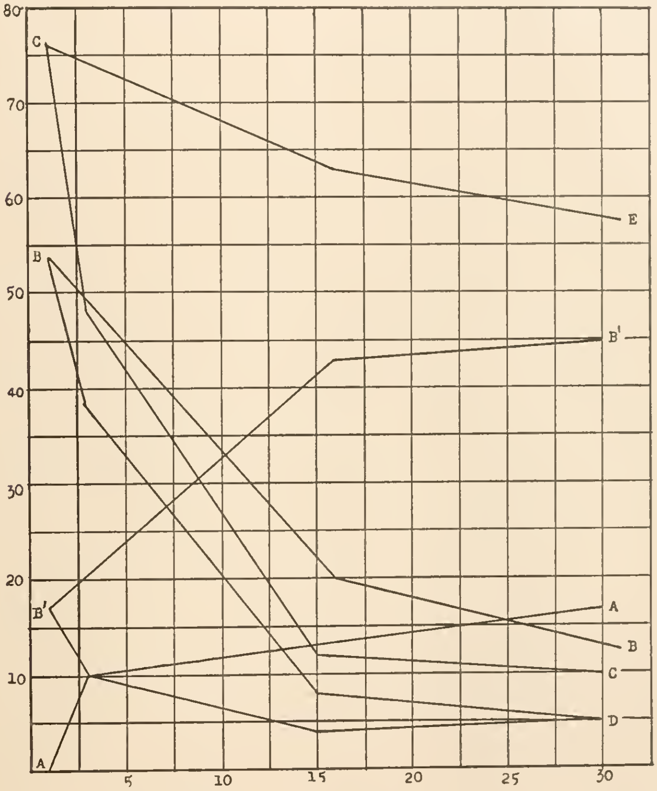

FIG. *A*. The effect of fertilizing eggs previously treated with potassium chloride. Data taken from Table VII. The abscissæ represent minutes, the ordinates the percentage of eggs developing to swimming larvæ.

time before fertilization (compare *CC*), but it is a decided drop. Breaking this curve up as before, to show the proportion of segmented and unsegmented swimmers,—*BB* represents the decrease in segmented swimmers, but *B′B′* shows a decided rise in proportion of unsegmented swimmers, although the total percentage of swimmers produced is less than in the fertilized control or in the lot subjected to KCl action for a brief time only (see Table VIII. fertilized after KCl 1 minute). In other words, it appears that in the eggs which have been subjected to a slight or slow KCl action the spermatozoön is unable to induce cleavage, so that we obtain many unsegmented swimmers,—while in eggs showing a greater or more rapid KCl action the changes are so great that the sperm cannot even induce differentiation, in such a doubly stimulated egg. It seems that the effect of the potassium chloride is a very decided one, which once having been established in any egg, inaugurates such changes in it that it is no longer capable of normal fertilization. (A few eggs may be able to withstand the double action.) The longer the potassium chloride acts the further the changes proceed, and the more abnormal is the larva resulting from fertilization after KCl. In short the eggs cannot endure this double stimulation any better than they can polyspermy.

These results, it seems to me, are exactly what would be expected. For if we consider the artificial stimulating agent as acting in somewhat the same way upon the egg that the sperm does, we would thus get in the egg some of the same sort of changes set up which would take place in a fertilized egg. If then we add sperm after this series is set going, the fertilization is not to be expected to be normal.

2. *Cold and Sperm.*—Eggs placed at a temperature of 9.5° C., and fertilized after removal to room temperature, showed considerable reduction in percentage of swimmers, especially segmented ones, the reduction increasing with length of time, and apparently more rapidly if the eggs were subjected to the continuous action of cold up to the time of fertilization than if an interval of time at ordinary room temperature was allowed to intervene between the application of the low temperature and the addition of the sperm. Here, as in KCl + sperm there is a ten-

dency for adverse conditions to interfere first with cleavage, and second to destroy the power of differentiation into swimmers also (see Table X.).

TABLE X.

Treatment.	Segmented Swimmers, Per Cent.	Unseg. Swimmers, Per Cent.	Total Per Cent.
Fertilized control	72.5	1.5	74
9.5° C. 30 min., then room temp. 15 min., then sperm	15	44	59
9.5° C. 1 hour, then sperm	0	12	12

3. *Excess of Oxygen in the Sea-water and Sperm.*—The effect of an excess of oxygen in the sea-water is here considered as a primary stimulus to artificial parthenogenesis and therefore included with the other experiments of fertilization supplementary to a physico-chemical parthenogenetic agent. When sperm were added to eggs which had been for varying times in oxygen-saturated sea-water, in one case the number of swimmers resulting in the experiment was reduced one half or more from that of the fertilized control. That the reduction was not due to length of time after removing the eggs and sperm from the animals was seen by making a fertilized control at each time when an experimental lot was fertilized (Table XI.).

TABLE XI.

Time in O_2 Sea-water.	Followed by Time in Sea-water before Fertilized.	Swimmers, Per Cent.		
		Segmented.	Unsegmented.	Total.
Control—0.	0.	44	22	66
16 min.	21 min.	8	25.5	33.5
Control—0.	37 min.	35.5	24	59.5
65 min.	1 min.	5	7	12
Control—0.	66 min.	37.5	27.5	65
120 min.	0.	12	5	17
Control—0.	120 min.	43	25.5	68.5

The sperm and eggs were evidently just as able to develop normally at the end of the experiment as at the beginning. Nevertheless the eggs which had been subjected to the action of an excess of oxygen showed a much lower per cent. of swimmers than the control, and the effect was greater with longer time of oxygen action,—results entirely comparable to those in the KCl + sperm experiments.

B. Combination of Two Physico-chemical Agents.—A number of different combinations were made, using potassium chloride with other physico-chemical agents, in the hope that some other agent added to the potassium chloride would induce cleavage. The method met with little success. In fact in many cases the results were similar, in their suppression of development, to those produced by using a parthenogenetic agent and sperm.

1. *Double Potassium Chloride.*—A weak potassium chloride solution (1 c.c. 2½ *M* KCl + 49 c.c. sea-water), a concentration suited to induce maturation, was allowed to act for four minutes. The eggs were then removed to sea-water where they remained 10, 20, 40, or 60 minutes. They were then placed in a second potassium chloride solution, (5 c.c. 2½ *M* KCl + 45 c.c. sea-water),—a solution usually suited to induce the formation of swimmers. The eggs were left in this second KCl from 9 minutes to 2 hours. Almost no swimmers were formed! Some changes had apparently been initiated by the first "dose" of the potassium chloride, which prevented the second "dose" from having the effect it would have been expected to have by itself. Here, as in the combining of artificial parthenogenesis and fertilization, the time factor is important,—that is, the length of time after the initiation of development determines the extent to which development shall have proceeded, and the farther it has proceeded, that is, the longer the time, the less possibility there is of another agent inducing its normal reaction in the egg.

2. *Oxygen and Potassium Chloride.*—In the experiments with oxygen and potassium chloride, application of oxygen excess to the eggs while they were in potassium chloride made the experiment less successful than with potassium chloride alone, whereas used after potassium chloride, oxygen-saturated sea-water induced the formation of a slightly larger percentage of swimmers than the potassium chloride alone. Moreover a set of experiments in which the oxygen content of the water was raised merely by running a continuous stream of the gas through the water for a short time before, and during the experiment, had as a result the formation of a number of very normal looking blastulæ (Fig. 8),

and the next morning several swimming, apparently segmented, larvæ. The sections show a number of segmented blastulæ, but no segmented swimmers. In this case the eggs were left in 3.5 c.c. of $2\frac{1}{2}$ M KCl + 96.5 c.c. sea-water until after the formation of the second polar body, and were then placed in the oxygen-saturated sea-water, where they were left from one half hour to four hours, approximately. Ordinary "KCl larvæ" were present in all cases, as well as the few segmented larvæ. Sections show considerable amitosis, and some pretty good di-polar spindles,—perhaps a better condition of mitotic figures, in general, than in ordinary KCl material,—and a few blastulæ, some practically normal, some abnormal. Many pseudo-blastulæ appear, *e. g.*, a large nucleated cell at one end of the larva, with a row of smaller cells at the other end separated from the large one centrally by a cavity. These smaller "cells" do not contain nuclei, but appear to have chromatin scattered in the cytoplasm. The experiment was performed on four different days, but only once did any considerable number of segmented individuals arise, unless the small, nucleated, isolated cells, appearing in one experiment, were due to cleavage followed by a falling apart of the blastomeres. I did not catch them in process of cleaving, so cannot be sure whether such cleavage was mitotic or mere fragmentation. Because of the infrequency of the result, I do not place much weight on it as a means of inducing cleavage unless in some way the results may be made more uniformly certain. However, the fact remains that oxygen-saturated sea-water used after the eggs have been subjected to the action of potassium chloride solution, appears to affect the eggs quite differently from a second "dose" of potassium chloride itself. It would appear that the oxygen has a somewhat different function to perform from that of the potassium chloride, and therefore the use of the one does not prevent the action of the other when one follows the other. But used *together* they are antagonistic.

3. *Heat and Potassium Chloride.*—As a companion result of the oxygen-saturated sea-water used after potassium chloride, may be given that obtained when heat and potassium chloride were used together. In both cases there was an increase in swimmers

above the number in potassium chloride alone. In the heat experiments the eggs were placed in sea-water, in a warm bath, the sea-water containing 2 per cent. or 7 per cent. of 2½ *M* KCl. At intervals varying from 5 minutes to 1½ hours they were removed to ordinary sea-water at room temperature. With heat alone segmented swimmers were obtained, but with the combination of the two agents, only typical "KCl" swimmers were to be found. Thus while the combination made the KCl experiment more successful, on the other hand it really interfered with the heat action.

4. *Cold and Potassium Chloride.* (Table XII.)—The action of cold combined with potassium chloride was to decrease the power of differentiation, but to increase polar body formation. 36.5 per cent. of the eggs in sea-water containing 2.5 per cent. 2½ *M* KCl at room temperature for 30 minutes, formed one or both polar bodies and 16.5 per cent. formed swimmers, unsegmented. Of eggs from the same lot, in KCl sea-water of equal strength but at a temperature of 9.5° C., 79 per cent. formed one or both polar bodies, but only 9 per cent. formed swimmers. The cold alone for 30 minutes caused no noticeable development, but applied for 60 minutes it brought about the formation of the first polar body in 47 per cent. of the eggs, but no swimmers (in this particular experiment). Thus the number of eggs extruding the first polar body was as much increased, approximately, by the combination of the two treatments, as the sum of the two effects each considered alone (KCl 36.5 per cent. and cold 47 per cent.,—KCl and cold together 79 per cent.). But the number of swimmers was decreased from the number induced by potassium chloride alone, although increased above that for cold alone, for in the latter case there were no swimmers produced. It would

Table XII.

Per Cent. of 2½ *M* KCl in Sea-water.	Temperature.	Time, Min.	Per Cent. Forming Polar Bodies.	Per Cent. Swimmers.
3.5	Room temp.	30	36.5	16.5
3.5	9.5° C.	30	79	9
None.	9.5° C.	30	0	0
None.	9.5° C.	30	47	0

seem that the development of the swimmers, then, was entirely due to the potassium chloride, but hindered by the low temperature. Cold alone, as noted earlier in the paper, may under favorable conditions lead to the formation of swimmers.

5. *De-aerated Sea-water and Potassium Chloride.*—Two sets of experiments were performed, to test the effect of potassium chloride in connection with de-aerated sea-water,—one set using the potassium chloride before, the other at the same time with, de-aerated sea-water. The results were not very different from those with potassium chloride alone. The percentage of eggs forming polar bodies under either treatment was about equal to that obtained with potassium chloride alone. No swimmers were formed as a result of the potassium chloride alone in this case, and none with the use of the combination, except in two cases, namely, 0.5 per cent. swimmers were formed in a lot of eggs left 1½ hours in de-aerated sea-water + 2 per cent. 2½ *M* KCl, and 3 per cent. swimmers were formed in a lot left over night in de-aerated sea-water after being in 2 per cent. 2½ *M* KCl 3 minutes. The swimmers were unsegmented.

6. *Potassium Chloride and Sea-water De-oxygenated by a Stream of Hydrogen.*—A stream of hydrogen was run through a flask or bottle of sea-water to remove the oxygen. A small amount of oxygen was doubtless present, however, entering with the eggs, etc. The eggs were placed in 3.5 per cent. of 2½ *M* KCl in sea-water, 45 minutes to one hour, where many formed either one or both polar bodies. They were then removed to the hydrogen sea-water, and remained there from 8 minutes to 3 hours, with a constant stream of hydrogen bubbling through the water. A very few looked suggestive of first or second cleavage. Next morning swimmers were found in all lots, particularly the three hour one. Some appeared abnormally segmented. Sections, however, show practically no normal cleavage, but one or two ciliated larvæ were found which were very abnormally cut up into cells. The rest were much like ordinary KCl material.

7. *Potassium Cyanide and Potassium Chloride.*—Many combinations of potassium cyanide and potassium chloride were tried. In these experiments the differentiation was in general poorer than with potassium chloride alone. A great deal of breaking

up occurred, and some possible cleavage, but probably not normal mitotic cleavage. In several of the experiments there was a suggestion in the living material of a small amount of early cleavage, but the next morning segmented swimmers were not to be found, except in one set of experiments, when one possible segmented larva appeared as a result of treatment with a solution of one drop of *n*/10 KCN in 100 c.c. sea-water for one hour, following 3.5 per cent. 2½ *M* KCl in sea-water for about 45 minutes,—and another among the eggs left in the same KCN solution for 2½ hours. Sections from this set of experiments show much breaking up into small round masses, which may be abnormal cells. The material is suggestive, but that is all. It may be that the treatment causes a slight amount of cleavage and some breaking up, but certainly rarely, if ever, like that of normal development. It is interesting to note that in a number of *Chætopterus* experiments many lots of eggs which in early stages are most suggestive of cleavage, in late stages show less differentiation than in some other experiments. It may be that unless the cleavage is normal, differentiation is more difficult with cleavage than without it.

8. *Sodium Chloride and Sodium Hydroxide.*—To 8 c.c. of 2½ *M* NaCl + 50 c.c. sea-water, was added either 0.2, 0.4, or 1.0 c.c. *n*/10 $NaOH_2$. The eggs were transferred to ordinary sea-water after 20, 50, 75 and 120 minutes. Swimmers appeared in all the dishes except the 120 minute lot, and the 1 c.c. *n*/10 NaOH lot for 20 minutes or 75 minutes. The best results were obtained from the 0.2 c.c. *n*/10 NaOH lot for 50 minutes. No cleavage was noted. Something resembling a large polar body was formed, similar to that seen in the sodium chloride treatment without sodium hydroxide.

V. DISCUSSION.

1. *Problem of the First Changes.*—The first changes in development are initiated by the entrance of the egg into sea-water from the ovary of the animal. This stage in the development consists in the break-down of the germinal vesicle, the migration of its contents to the animal pole, and the formation of the metaphase of the first maturation spindle. The freeing of the vitelline membrane from the surface of the egg, with the accompanying

formation of the peri-vitelline space, takes place at the same time. The experiments performed with reference to a determination of the factors operating to bring about these phenomena were too scattered to do more than suggest further lines of work. A few of the findings are interesting, however, in their possible bearing on the behavior of other eggs which do not go through their development in precisely the same way.

No membrane forms in *Chætopterus* as a result of fertilization, but a space arises between the egg and the vitelline membrane already present, as soon as the egg is put into sea-water. The cortical changes involved are comparable to those concerned in the formation of the fertilization membrane in Echinids, yet the development in *Chætopterus* ceases with the formation of the first maturation spindle, and all eggs, unless given some further treatment, undergo degenerative changes. The cortical changes which accompany the formation of the membrane induce only a brief period of activity, and do not lead, of themselves, to development. Loeb ('09) has said that in the sea-urchin in many cases the cytolysis which is started at membrane formation leads to the death of the egg unless checked by a second agent. It is possible that in *Chætopterus* also the death of the egg after membrane formation is due to cytolysis set up at the time of membrane formation. But it seems more probable that the changes which accompany membrane formation in *Chætopterus* represent only a certain part of those which accompany the process in Echinids, and therefore the same results do not follow in both cases.

The experiments indicate that various salts have a definite effect upon the eggs,—*e. g.*, in $n/2$ sodium chloride or $n/2$ potassium chloride the eggs form apparently normal spindles, whereas in $n/2$ calcium chloride and $n/2$ magnesium chloride they do not. Magnesium chloride induces the formation of fairly normal membranes, whereas neither potassium chloride, sodium chloride nor calcium chloride bring about this result. But neutral paraffin oil also induces normal spindles, and *Chætopterus* body fluid gives normal results in all respects, in a certain proportion of eggs. Therefore it is evident that no one specific chemical agent is necessary for this first reaction of the egg, but that it may be induced in a number of different ways. It is possible that the changes are induced by some physical factor, such as an increase in the permeability of the egg membrane or surface caused by a change in the osmotic pressure of the surrounding medium. There is very little evidence for this so far, however.

2. *Problem of Maturation.*—In the artificial parthenogenesis of *Chætopterus* development may proceed whether one or both polar bodies have been extruded, or both have been retained within the egg. This phenomenon has been noted in other forms. Lefevre ('07) found it to be so for *Thalassema mellita*, Delage ('02) for *Asterias*, Scott ('06) for *Amphitrite*, Kostanecki ('11) for *Mactra*, Treadwell ('02) for *Podarke*, and Morgan ('00) and Lillie ('06) for *Chætopterus.*

The cytological questions of spindles and chromosomes have not been taken up in many cases. Lefevre ('07) found in *Thalassema* that when only the first polar body was cast out of the egg, the second polar spindle was to be seen deep within the cytoplasm,—as a result of which two nuclei might be formed in the egg and fuse to form the cleavage nucleus. He was unable to get an accurate chromosome count but was able to ascertain that more than the normal reduced number appeared in later stages. When no polar body was thrown off he found in most cases only one maturation division and this took place within the egg, with subsequent union of nuclei. These observations are similar to certain phenomena which I have seen in *Chætopterus.* In this egg also the second polar spindle might often be seen lying deep within the egg, and forming two nuclei which united. Counts of chromosomes in later stages indicated that at least an approximation of the normal $2n$ number was present. Similarly when no polar body was thrown out two nuclei were formed which united in certain cases.

In *Amphitrite* Scott ('06) found quite a different case, apparently. Here the chromatin of the first or second polar body might be thrown off in a mass in the cytoplasm. In *Asterias* Delage ('01) observed 18 chromosomes, the $2n$ number, when but one polar body had been thrown off. In *Chætopterus* Morgan ('00) found two nuclei when the second polar body had not been extruded. In *Mactra* Kostanecki ('11) observed that whether one, two, or no polar bodies had been thrown off, cleavage of chromosomes and nuclei, unaccompanied by cell-division, occurred, followed by later cell-division with a regulation of the nucleo-plasmic relation.

There is very little in the literature relative to the physiological cause of polar body formation in particular, aside from the general question of parthenogenesis, but a few theories with regard to the retention or expulsion of the polar bodies and the consequences to the egg, may be mentioned. Scott ('06) suggests for *Amphitrite* that potassium leads to the taking up of water by the egg, that this tends to prevent the normal collapse of the egg, and thus also interferes with the formation of polar bodies. In this connection he calls attention to the fact that in *Chætopterus* maturation is hindered by strong KCl, but not by weak potassium chloride.

Delage ('01) at one time thought that the retention of the second polar body within the egg was an important factor in the parthenogenesis of the star-fish egg, "en fournissant à l'œuf les éléments qui lui manquent," but later he changed this opinion largely, his later view ('02) being that parthenogenetic development depends on the arresting of maturation at some point when the nucleus is in the mitotic phase. When the egg is reawakened, as it were, it proceeds to cleavage. In this way only is artificial parthenogenesis dependent upon polar body formation.

Loeb ('02) stated the necessity of free oxygen and OH-ions in the sea-water in certain concentration to cause or accelerate maturation in *Asterias forbesii*. He was able to prevent maturation by a lack of oxygen or by adding acid or potassium cyanide to the sea-water. The addition of sodium hydroxide or benzol was found to induce maturation. Wolfsohn ('07) observed similarly for *Acmæa* that sodium hydroxide or fat solvents brought about maturation, and that the passage of a stream of hydrogen through the water inhibited it. Also "by stopping the oxidative processes in the egg through the presence of potassium cyanide, maturation can be inhibited." In these cases, then, it would appear that maturation is bound up with oxidation processes of some sort in the egg.

In *Chætopterus* the matter is rather different. Not only is lack of oxygen not prohibitive of maturation, but it has been determined that a solution of potassium cyanide, or the use of sea-water de-aerated by boiling, actually stimulated the eggs to form the polar bodies, and that, moreover, while the eggs were still in the solutions. In *Chætopterus*, then, may it not be that maturation is a phenomenon concerned mainly with hydrolyses? It seems to be generally agreed that hydrolyses can proceed in lack of oxygen or in presence of potassium cyanide. The action of cold used with potassium chloride is interesting in this connection. The percentage of eggs forming polar bodies in this case was greatly increased over the percentage of maturation induced by potassium chloride alone. If cold suppresses oxidative processes, this may be the reason for the increase in maturation percentage. On the other hand, eggs subjected to the action of heat do not form the polar bodies while in the warm water, and in many cases the second polar body is never extruded, even though development proceeds to the formation of swimming larvæ. Now if heat, as is generally supposed to be the case, increases the rate of all reactions, it must increase both the hydrolyses and the oxidations. Here, then, there should be a stimulus to maturation, and this is in fact seen in certain eggs which do form one or both polar bodies. But there is also either an active suppression of maturation or a lack of stimulus to it in the *majority* of eggs, for most of them form only one, or neither, of the polar bodies. Where oxidations are increased, then, there is a tendency to omit maturation. As another bit of evidence, we may include the observation that an excess of oxygen used at the same time with potassium chloride somewhat lessened the percentage of swimmers, but used *after* potassium chloride, increased it. Here again, apparently, excess of oxygen at the very beginning of development is not good. The fact that oxygen-saturated sea-water will induce a small percentage of eggs to form polar bodies, may seem contradictory. But it is possible that here we have a quite different set of reactions leading to some of the same results. For example the oxygen excess may serve to stimulate some reaction other than oxidation, only gradually bringing about an increase in the oxidation rate. Thus it may be that the reactions which are set in motion go on with a low oxidation rate for a time, and hence are accompanied by maturation.

R. S. Lillie ('08) says of the star-fish egg, "Suppression of oxidative combined with acceleration of hydrolytic and reducing processes is indicated as a condition of the initiation process in these eggs." Loeb ('09), in commenting on Lillie's conclusion, suggests that suppression of oxidation in the egg has its beneficial effect not by allowing anaerobic processes which in themselves are of importance to the development, but by giving the egg time to recover from the injurious effects of membrane formation before proceding to further development.

Another body of observations given in the experiments should be mentioned here. With nearly all agents employed, the longer exposure to the agent or the higher concentration of it, are prejudicial to maturation. On the other hand, production of swimmers occurs in larger percentages with high concentration and long exposure. If, as suggested, the course of development is composed of two rather distinct sets of reactions, the one accompanying maturation and concerned mainly with hydrolyses, the other resulting in differentiation and requiring oxidation processes, then it seems reasonable to suppose that an agent which calls out both maturation and differentiation calls out both sets of reactions, the one gaining force a little later than the other. The two processes take place in their normal relation when a certain concentration and time is used, but when this concentration or time is increased, the normal time relation between the two is interfered with and the second reaction arises before the first is completed. That is, the hydrolyses become obscured by the oxidative processes which always accompany differentiation. If these oxidative processes be of high enough rate to start up the processes that lead to differentiation more rapidly than the hydrolyses are forwarding the processes leading to maturation, then obviously the later developmental phenomena may come in and cut short the maturation phenomena.[1]

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[1] This actually seems to be the case when the second maturation spindle is apparently drawn down toward the center of the egg (as the female pronucleus would be, normally) before mitosis has been completed and the second polar body thrown off.

A second possibility to account for the effect of the potassium cyanide and the de-aerated sea-water has been suggested, namely, that these agents act as stimulants which are followed by a depressing effect, one acting directly, the other through allowing an accumulation of carbon dioxide. According to this hypothesis potassium cyanide may be regarded as a protoplasmic poison which first stimulates, then depresses. It is to be remembered in this connection that no development to swimmers follows maturation brought about by potassium cyanide or by de-aerated sea-water. The de-aerated sea-water under this theory may be thought to act indirectly, by permitting the accumulation of carbon dioxide given off by the egg as a result of the intracellular oxidations. No more carbon dioxide would be given off supposedly, than under normal conditions, but in this case there is no oxygen to keep the balance of processes normal. The accumulation of carbon dioxide might then act as a stimulus, as it is known to do in other cases. Carbon dioxide above a certain amount, however, is toxic to an organism, and after it has reached this amount with the eggs it may act as a narcotic, inhibiting further development. A simple experiment to test this might easily be performed with a stream of carbon dioxide. Thus the potassium cyanide and carbon dioxide would, according to this hypothesis, act first as stimulants, and then as depressants. Just what physiological processes are involved in such "stimulation" is unknown.

3. *Problem of Differentiation.*—The term differentiation is used here to denote the developmental changes which occur after maturation and which lead, when complete, to the production of swimmers. Cleavage may be omitted in the process of differentiation. Cleavage is, of course, essential to the production of *normal* larvæ, but certain differentiations may go on without it, even to the production of swimming larvæ.

Differentiation may be induced in *Chætopterus* by a varied list of agents, both physical and chemical. Nearly every agent tried induced either differentiation or maturation or both, and it is probable that the list of possibilities is very extensive indeed. Besides the agents which I have mentioned in this paper, Loeb ('01) has brought about development in *Chætopterus* by the use of magnesium chloride, calcium chloride, cane sugar, potassium bromide, potassium nitrate and potassium sulphate. The egg must be in a very labile state, such that almost any slight impetus will force it from its condition of unstable equilibrium.

There are, however, indications of specific needs of the egg, satisfied more or less completely by the various methods of treatment. One of the most obvious needs, it seems to me, is for oxygen. In this respect, as before stated, there shows up a distinct difference between the maturation and differentiation processes. Although maturation is called out in lack of oxygen or in low rate of oxidation, differentiation, at least to the point of forming swimmers, is often interfered with by these conditions. A brief suppression of oxidation by potassium cyanide, following potassium chloride interfered with the production of swimmers in *Chætopterus*. On the other hand, increased supply of oxygen in the sea-water after treatment with potassium chloride led to some of the best results obtained. By far the most normal results were obtained from the application of heat for a stated length of time. Although heat may act in many ways, it is very probable that one of its effects is to increase the rate of oxidations within the egg. It is also well-known that heat increases the permeability of certain membranes to oxygen. It may be, therefore, that heat is an important aid to the oxidations necessary to differentiation. Greater oxygen pressure in the sea-water surrounding the egg will supply the need to a certain extent, but not so surely as will heat.

In plant seeds it has been found by Crocker ('06) in several cases, that the factor which causes the dormant period between the formation of the embryo and the germination of the seed is an insufficiency of oxygen in the seed due to the semi-permeability of a membrane of the seed to oxygen. When greater oxygen pressure was supplied the delayed germination proceeded. In one case heat increased the permeability of the membrane to oxygen and brought about the same result as did increased oxygen pressure in the environment of the seed. The seed in its dormant condition is of course different from the unfertilized egg in that the embryo is already formed, but the conditions that determine dormancy and the awakening from dormancy may not be very different in the two cases. In the case of several animals the same agents which will arouse a dormant seed will arouse a dormant egg,—that is, acids, bases, heat, increased oxygen pressure, etc. The effect of these agents in a number of seeds has been traced by Crocker and others, to the effect of the agents in making the seed membranes more permeable to water or to oxygen, according as the need might be. It would be going too far to suggest that the egg is always dormant for the same simple reason, an impermeability of its membrane to water or to oxygen,—but certainly some physical or chemical change is necessary, which may be induced either by the sperm or by some non-living physical or chemical agent,—the problem being to find just the need of the egg, that is, what is lacking to cause it to continue its development. In the case of *Chætopterus* one of the factors lacking for differentiation would seem to be sufficient oxygen or sufficiently high rate of oxidation.

The question of the semi-permeability of the plasma-membrane seems to be a very vital one. R. S. Lillie ('11), in his study of artificial parthenogenesis in *Arbacia*, states that the critical change in the egg to which the initiation of cleavage is due is a well-marked and rapid increase of the permeability of the plasma-membrane. He has lined up a number of salts whose power to induce development runs parallel to their power to increase membrane permeability. Among these salts are the chlorides, bromides, and nitrates. The potassium salts of all of these, and the sodium, calcium, and magnesium salt of the chloride, have been found to induce development in *Chætopterus*, and it seems probable that they may exert the same sort of effect on this egg that they do on *Arbacia*. Lillie ties up the change in permeability with a change of electric polarization ('12), and a sudden diminution of the resistance to the progress of the oxidative energy-yielding reaction, brought about by allowing the escape of the products of oxidation,—particularly carbon dioxide ('09).

It is a well-known fact that developing eggs need much more oxygen than resting, undeveloping ones. Various theories have been brought up to account for this need. Loeb ('09) considers that certain oxidative processes are advantageous in checking the cytolysis set up in the initiation of development,—which cytolysis goes on to the destruction of the egg unless checked. Child ('11) suggests that the egg is a cell overloaded with food-stuffs and structural obstacles to metabolism,—that therefore increased metabolism is necessary for its further development. To quote him more exactly, "Physiologically the gametes are in the extreme stages of senescence and can be saved from death only by some regulatory change, which permits increased metabolism, and more specifically probably increased oxidations and syntheses."

All the facts seem to point to the conclusion that the reactions concerned in maturation and those in differentiation are somewhat different. F. R. Lillie ('11) has shown for *Nereis* that there may be a separation of the stimuli to the two processes even in the case of fertilization,—maturation being induced by contact of the sperm with the egg,—whereas differentiation does not follow except with penetration of the sperm within the egg. Loeb ('09) has noted in *Asterias forbesii* that the agent which causes development may hinder maturation, and has suggested that in eggs in which the entrance of the sperm calls out both maturation and development, the same chemical agent may call out both processes. This proves true for *Chætopterus* within certain limits, as already noted, but at the same time the evidence points to the presence of two different sets of reactions for the two processes.

4. *Problem of Cleavage.*—Inasmuch as most agents call out differentiation without cleavage in *Chætopterus*, and only two so far have induced the formation of segmented larvæ, there is the possibility of studying some of the fundamental questions of cell-division here.

Most writers on cell-division have attacked it mainly from the morphological side, making it a question of asters and centrosomes. Cleavage, according to Boveri, is a matter of the presence of the centrosome and of its normal activity. In *Chætopterus* centrosomes may be present, but they do not lead to cleavage necessarily. They may divide to form a group of centrosomes in the astrosphere, or they may not be visible at all in the aster. The mitotic figure is extremely abnormal in the KCl material, and cleavage is absent. There is certainly a connection between the two facts of abnormal mitotic figure and failure of cleavage, whether one or the other is causal, or whether both are caused by the same thing and merely accompany each other, as many think.

It is certain also that the presence of the normal number of chromosomes in the cleavage nucleus does not insure cleavage. In many cases where only one polar body is extruded the $2n$ number of chromosomes is present, but cleavage does not follow. In the heat experiments, which show the best results that I have obtained, only one polar body, or none, was thrown out, and the $2n$ number appears to be present therefore. Here cleavage was obtained.

According to R. S. Lillie ('11) the mitotic figure represents an electric field of force, formed because of the more or less sudden increase in permeability at two points on the egg surface, to ions of the opposite sign from those already determining the sign of the egg protoplasm. In his experiments with *Arbacia* eggs he found that it was necessary not only to increase the permeability of the membrane in order to induce cleavage, but also to increase it so that the inflow might take place at a certain rate. If this interpretation of cleavage be correct, then it may be that in the case of *Chætopterus* the agents which cause differentiation without cleavage are such as to cause increased permeability, but not with sufficient rapidity to lead to cleavage. Heat, on the other hand, may act by causing more sudden increase.

If, on the contrary, cleavage be thought of as conditioned, not by electrical phenomena, but by a certain viscosity of the egg, as suggested by Loeb ('92) when he says that the checking of cell-division in a hypertonic solution may be due to a raising of the viscosity of the protoplasm as a result of loss of water, then it seems possible that KCl and other agents which are thought to extract water from the egg, extract it to so great a degree that the egg is unable to form the cleavage plates because of too great viscosity.

Normal cleavage requires that certain processes shall take place in correlation. It was evident from the KCl material that reactions were taking place in the egg which determined chromosomal cleavage, and cytoplasmic cleavage. But the two were not correlated. The two processes did not occur at the same time, nor in any morphological relation to one another. The problem of inducing both processes to go on at the same rate and in morphological correlation is met by the heat treatment. It seems very possible that the question of permeability is concerned not only with the external egg membranes, but that a concentric system of semi-permeable membranes or regions, involving, perhaps, alveolar and nuclear membranes, are important factors. F. R. Lillie ('08) has shown definitely that the ground substance of the eggs of *Chætopterus* consists of four concentric layers, differing from one another in density and in aggregation of characteristic granules. If these regions and the various membranes must all become permeable and in such manner that entrance and exit of substances can take place at a certain rate, in order that reactions which should be correlated may be set up at approximately the same time, it seems very probable that the power of heat in inducing the most normal results so far obtained with *Chætopterus* is due in part to the fact that its effect is very quickly felt throughout the egg. (Other agents doubtless permeate more slowly.) In this way regions and membranes deep within the egg may be affected at approximately the same time as the more superficial ones, and thus the reactions which arise, may arise in correlation, and the nucleoplasmic relations normal for development may be set up in the egg. F. R. Lillie ('12) has concluded from his studies on the effects of partial fertilization in *Nereis* that "the establishment of normal metabolic interchange between the nucleus and the cytoplasm must be regarded as a fundamental function in artificial parthenogenesis." Any agent, then, which will affect the inner regions of the egg at the same time as the outer, may be expected to have a great advantage over one which is slow to penetrate—in that it has a much better chance of establishing normal correlations and therefore normal metabolic interchange.

5. *Artificial Parthenogenesis Supplemented by Fertilization.*—Loeb ('07) states that sea-urchin eggs fertilized after treatment with hypertonic sea-water do not develop normally, but often show multipolar spindles.

Herbst ('07) found that when he fertilized eggs of *Sphærechinus* after subjecting them to the action of butyric acid he got polyspermy and abnormal larvæ, but that this effect followed only when a certain amount of time was allowed to elapse after the application of the acid, before fertilization. With *Chætopterus* it may be said that in general any agent which induced artificial parthenogenesis interfered with normal development when fertilization was used after the artificial parthenogenetic agent. The injurious effects were greater, usually, with increased time allowed for the action of the parthenogenetic agent before fertilization, but very brief action also interfered with normal fertilization. It seems to me that these facts furnish evidence that the development induced by an artificial parthenogenetic agent is similar in its working to that induced by a spermatozoan. If the primary effect of fertilization or of artificial stimulation is regarded either as an increase in the permeability of the egg, or a starting up of cytolysis, it seems reasonable to suppose that the use of the two agents in the same egg should increase the permeability to an excessive degree or cause too rapid or extensive cytolysis, and therefore produce abnormal results. The time factor also adds its effect, in that the farther development has proceeded before the application of a second agent suited to initiate development, the more difficult it is for such an agent to produce its normal effect.

6. *Combination of Two Physico-chemical Agents.*—When we consider the combination of two physico-chemical agents we have a somewhat different proposition, although essentially the same. In this case the physico-chemical agents may or may not duplicate each other in reaction, according to their nature. If they do act as duplicates their combination appears to lead to injurious results, as in the case of adding sperm to artificial parthenogenesis. For instance when one solution of potassium chloride was followed after an interval by another solution of potassium chloride, practically no development followed. Maturation was initiated as a result of the first treatment, thus showing that the solution was effective, but when a second "dose" was applied it was unable to produce its usual effect,—indicating that the reaction had already proceeded to a point where a

stimulus of the same sort as that which initiated the development could no longer be effective. Duplicating the first stimulus would do no good. The time for the effective application of potassium chloride had passed. A stimulus of a different sort was needed.

When the action of the one agent supplements that of the other, however, the success of the experiment is increased. For example, potassum chloride added to heat reduced the percentage of segmented swimmers below the record for heat alone, but it increased the percentage of unsegmented swimmers above that for potassium chloride alone. As will be recalled, potassium chloride alone does not induce cleavage, but heat does do so. In this case, then, it is evident that heat is the better agent, since it induces a more normal result. It seems probable that the reactions brought about by the potassium chloride are incomplete in some way. Therefore when the two are applied together the effect of the potassium chloride is heightened, because some of the incomplete reactions are completed or supplied by the heat, whereas the heat effect is lessened because its working is fairly complete in itself, and is only injured by duplicating.

Summary of Discussion.

It is quite impossible in the present state of uncertain knowledge with regard to physiological causes and effects, as well as in view of the small number of the experiments brought forward in this paper, to draw any definitive conclusions as to the causes of the initiation of development in *Chætopterus*. I should like merely to summarize certain suggestions which seem to me most in line with the findings.

1. The great variety of agents which will induce the development of *Chætopterus* eggs indicates that the egg is in a very labile state of equilibrium, and that the same results may be reached by different processes.

2. Development may be resolved into a separable series of steps. A graded series of stimuli can be determined by which development may be made to stop at almost any step in the process.

3. The cortical changes which accompany membrane formation

in *Chætopterus* are associated with a very brief period of activity and are not sufficient of themselves to induce development.

4. Development to swimming larvæ may take place whether one or both polar bodies are extruded, or if both are retained within the egg.

5. Maturation and differentiation may be controlled by different conditions, indicating more or less distinct sets of reactions for the two processes.

6. Maturation may go on in reduced oxygen supply, or in suppression of oxidation by potassium cyanide. Hydrolyses are probably concerned in maturation, therefore, or other processes which can take place with very low rate of oxidation.

7. High rate of oxidation interferes with maturation.

8. Differentiation requires an increased supply of oxygen over that contained in the resting, unfertilized egg.

9. Differentiation involves oxidation. Suppression of oxidation by potassium cyanide, even for a brief time, during the differentiation period, hinders differentiation.

10. Excess of oxygen in the sea-water after the completion of maturation may induce cleavage.

11. The effect of heat in inducing cleavage is probably due in part to the intake of more oxygen by the egg, owing to increased permeability of the membrane, also to the increase in rate of oxidations.

12. Cleavage requires that the normal nucleo-plasmic relations for development shall be set up, such that reactions may take place in correlation. This is attained by the action of heat which affects all regions and membranes of the egg at approximately the same time.

13. The use of an artificial parthenogenetic agent before fertilization is prejudicial to normal development.

14. The use of two physico-chemical agents to induce artificial parthenogenesis suppresses the development called out by either, unless the action of one is supplementary to that of the other.

I wish to acknowledge my indebtedness to Professor F. R. Lillie, of the University of Chicago, for the use of his *Chætopterus* material, and for the many kind and very helpful suggestions given during the progress of my work.

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EXPLANATION OF PLATE I.

All figures were drawn with camera lucida with Leitz apochromat 2 mm. oil immersion objective, and Zeiss No. 6 compensating ocular, except Fig. 1 for which compensating ocular No. 12 was used.

FIG. 1. Egg fixed after remaining 30 minutes in 7.5 c.c. of 2½ *M* KCl + 92.5 c.c. of sea-water. The second maturation spindle is seen lying deep within the cytoplasm of the egg. Two chromosomal vesicles are shown at either end of the spindle. (It is entirely normal for these vesicles to arise in the formation of the nuclei after division.)

FIG. 2. Egg fixed after 35 minutes in 3.5 c.c. 2½ *M* KCl + 96.5 c.c. sea-water. The second polar nucleus and female pronucleus are about to unite to form the cleavage nucleus. The first polar body was seen in another section.

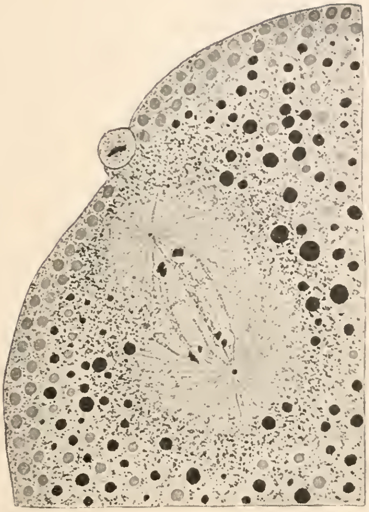
FIG. 3. Egg remained in 7.5 c.c. 2½ *M* KCl + 92.5 c.c. sea-water 60 minutes. Fixed five hours after the beginning of the experiment. Rods and granules of chromatin are seen migrating out from the nucleus into the cytoplasm.

FIG. 4. Egg treated as described for Fig. 3. Rods of chromatin previously cast out of the nucleus are seen lying in the cytoplasm. The nucleus is in the achromatic state.

FIG. 5. Egg remained in 7.5 c.c. 2½ *M* KCl + 92.5 c.c. sea-water 60 minutes. Fixed 14 hours after the beginning of the experiment. The figure represents an egg in the process of becoming multi-nucleate. Nuclei appear to be "condensing" from a large mass of scattered granules. Two polar bodies are shown.

FIG. 6. Multi-nucleate, unicellular, swimming larva. Egg treated with 3.5 c.c. 2½ *M* KCl + 96.5 c.c. sea-water 45 minutes. Fixed 14 hours and 15 minutes after the beginning of the experiment. A circle of nuclei surround a mass of yolk material and cytoplasmic granules.

PLATE I.



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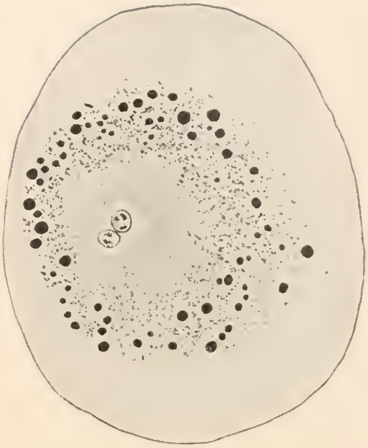

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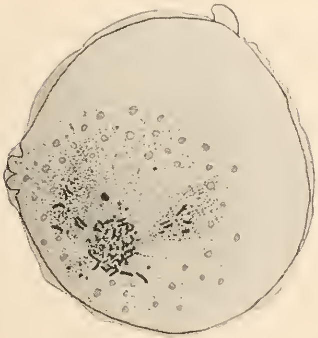

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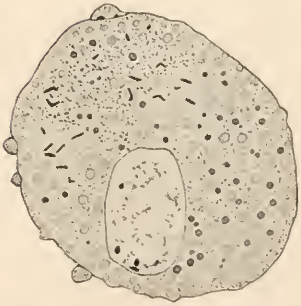

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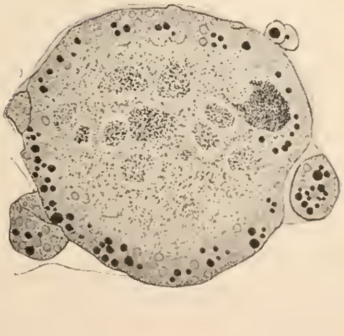

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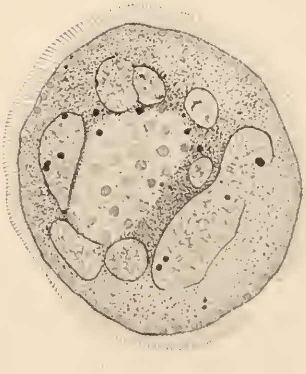

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HARRIETT M. ALLYN.

Explanation of Plate II.

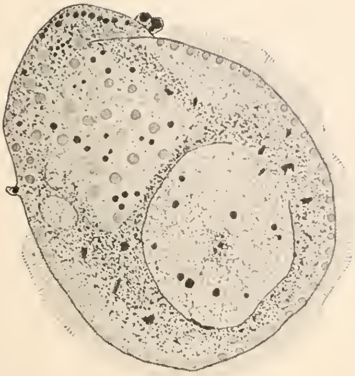
Fig. 7. Uninucleate, unicellular, swimming larva. Treated as described for Fig. 6. One or two very small nuclei and several ragged masses of chromatin are seen outside the main nucleus. The yolk mass is chiefly in the half of the egg nearer the animal pole. Two polar bodies have been thrown out.

Fig. 8. Blastula. Egg treated with 3.5 c.c. $2\frac{1}{2}\ M$ KCl + 96.5 c.c. sea-water until the formation of the second polar body, then with sea-water through which a constant current of oxygen was passing. Fixed 4 hours after the beginning of the experiment.

Fig. 9. Four cells of a six-celled specimen which had been subjected to sea-water heated to 32.5° C.–34.5° C. for 40 minutes. Fixed 3 hours and 15 minutes after the beginning of the experiment.

Fig. 10. Blastula. Egg treated as described for Fig. 9. Fixed 7 hours and 5 minutes after the beginning of the experiment.

Fig. 11. Larva. Egg subjected to sea-water heated to 33° C.–34.2° C. for 42 minutes. Fixed 21 hours and 16 minutes after the beginning of the experiment. Other larvæ of similar appearance were ciliated.



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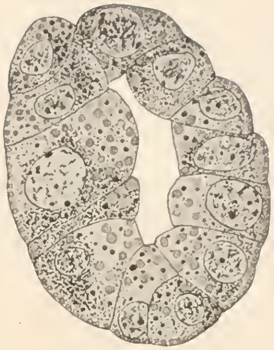

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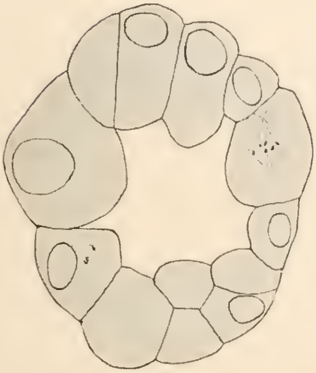

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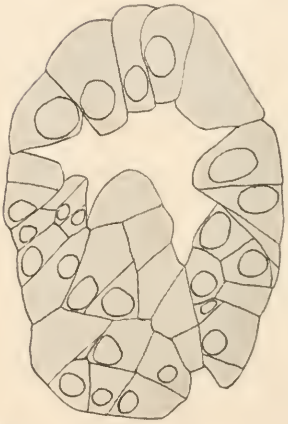

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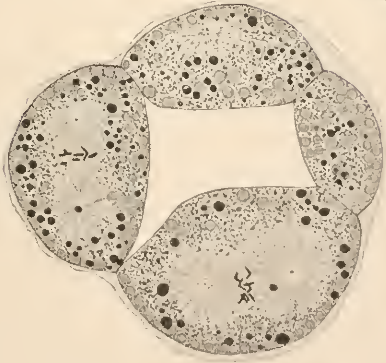

9

HARRIETT M. ALLYN.

Vol. XXIII. January, 1913. No. 2

BIOLOGICAL BULLETIN

STUDIES IN THE PRODUCTION OF GRAFTED EMBRYOS.

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Introduction 73
Methods Used to Agglutinate Eggs 74
Examination of the Effect of Treatment upon Development 75
The Early Development of Agglutinated Eggs 82
The Development of Agglutinated and Fused Gastrulæ 88
The Development of Agglutinated and Fused Plutei 93
The Development of Individual Clusters 97
Summary 98
Bibliography 100

INTRODUCTION.

It is now generally known that the blastomeres of certain kinds of eggs may, after their complete separation, develop into small though otherwise perfect, larvæ. The reverse experiment of reuniting partially separated or even completely separated blastomeres has also been successfully performed. These experiments demonstrated that it is not only possible to derive two embryos from a single egg, but that two or more blastomeres may be more or less recombined into one organism. These results suggested the possibility of grafting not only the blastomeres, but the eggs themselves. Metchnikoff ('86), Morgan ('95), Zur Strassen ('98), Herbst ('00), discovered and described embryos and larvæ which indicated a grafting of several eggs.

The first successful attempt to graft eggs together was made by Driesch in 1896 with the eggs of various European echinoderms. In the course of several years' experimentation, he perfected the method by which he produced agglutinated and fused eggs, about twenty to the thousand. More recently,

H. V. Wilson ('11), Müller ('11), and others demonstrated that somatic cells could also be fused.

Several investigators have repeated Driesch's experiments with the echinoderms found on this side of the Atlantic but without success. By slightly modifying Driesch's method, I finally succeeded in agglutinating and fusing the eggs of *Arbacia punctulata* at Woods Hole, Mass., in relatively large numbers, ten to forty in every hundred.

In this paper, I propose to give a detailed account of the method used in successfully fusing *Arbacia* eggs, to state briefly the effect of the treatment upon normal development, and to describe some of the agglutinated and fused embryos and larvæ. Since Driesch has given so full, so clear and accurate an account of fused larvæ, I will in this paper emphasize the earlier developmental stages and state but briefly in how far the *Arbacia* larvæ are like or unlike the European fusions described by Driesch.

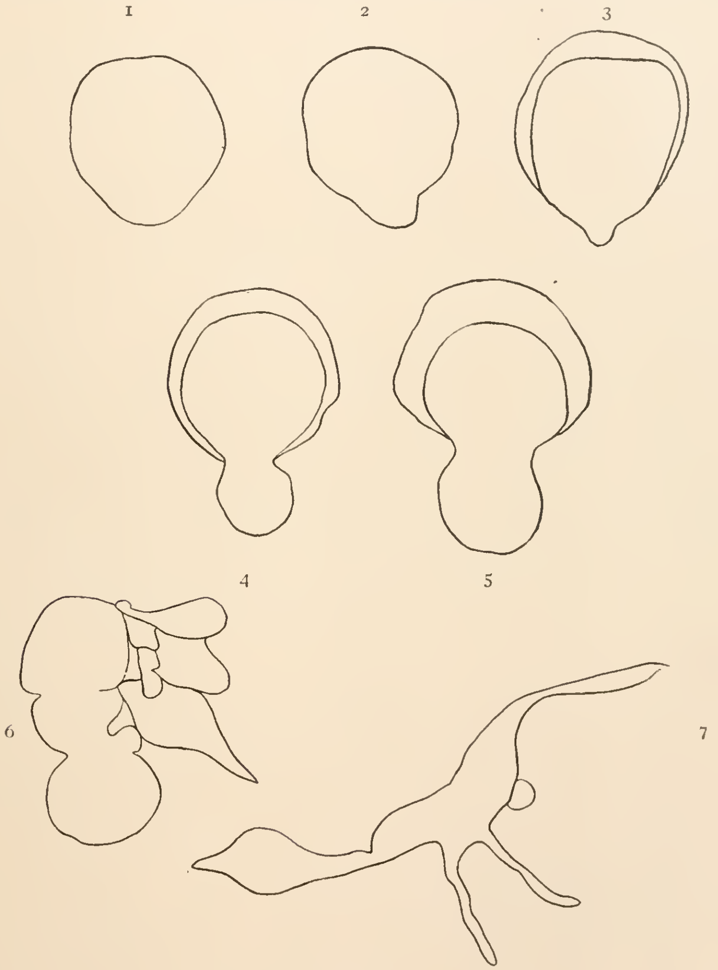
Method Used to Agglutinate Eggs.

The eggs of *Arbacia punctulata* were shaken violently two to three minutes after fertilization so as to remove their fertilization membranes. They were then placed in a calcium-free sea water (van't Hoff formula) prepared with copper or glass distilled water. To this solution four to twelve drops of 0.5 per cent. NaOH were added to every 200 c.c. of the solution. The eggs remained in this alkaline solution for varying periods, as a rule not longer than the first cleavage. Up to this point the method is the one used by Driesch, a method which failed altogether to agglutinate the *Arbacia* eggs. They were then transferred to narrow bore tubes, about 1/8 inch inside diameter, and centrifuged for three to five minutes at about 30 revolutions per minute, and finally placed in sea water. The cultures so treated later contained the agglutinated and fused embryos and larvæ.

As it seemed probable that the violence of the treatment might produce atypic results, a preliminary examination was made to ascertain to what extent anomalies were present in the cultures and how far these were the result of the technique used.

Examination of the Effect of the Treatment upon Development.

Shaking of the Eggs.—When the eggs were shaken about two minutes after fertilization, the fertilization membranes were cast off most of the eggs. When the eggs were shaken four or more minutes after fertilization, the membranes were removed with difficulty and the eggs were considerably distorted and otherwise injured. When shaken less than one and a half



Figs. 1–7.

minutes after fertilization, many did not segment at all or cleavage was irregular. It was evident that when eggs were shaken about two minutes after fertilization, the fertilization membranes were cast off most readily, and with the least disturbance to subsequent development.

The minimum shaking found necessary to remove the fertilization membranes from all or nearly all the eggs was about one and a half minutes. Figs. 1 and 2, drawn from life immediately after the shaking, represent the eggs freed from their membranes. In Figs. 3, 4 and 5, the eggs have only partially protruded from their membranes. Such extruded parts varied considerably in size, and were often equal to the part within the membrane, Fig. 5. Such "extra-ovates" were the same in kind, as those produced by Loeb' 94, with dilute solutions of sea water. When the egg was divided into two equal or nearly equal parts, twin embryos were formed which often reunited into a more or less single larva. When the extra-ovate was small, as in Figs. 3 and 4, it was most frequently sloughed off and developed into an atypic blastula or, in some instances, an atypic gastrula. Practically all the eggs were elongated but this change of shape was only temporary, and subsequent development was normal.

The rate of development was unaffected by the treatment. This may be seen in Table I. in which the rate of development in each culture is compared with its corresponding control. It is seen that in certain of the experiments the eggs developed more

TABLE I.

The eggs were violently shaken for about two minutes. Their rate of development when compared with the controls showed no material difference.

No.	Result.
20	Shaken lot developed at about the same rate as the controls. Many never reached the pluteus stage.
12*a*	Shaken lot never reached the pluteus stage.
b	Shaken lot behaved like the controls.
37	Shaken eggs developed faster, and more of them reached the pluteus stage, than the controls.
15	Shaken eggs were like the controls, lived longer, but contained more atypic larvæ.
14	Shaken and control lots the same.
25	Shaken and control lots the same. More small-sized larvæ in shaken lot.
34	Shaken and control lots the same. More small-sized larvæ in shaken culture.

slowly or more quickly than its control, but taken altogether the rate of development was clearly unaffected by the shaking; and irregular development was absent, except for the larger number of small atypic blastulæ and gastrulæ arising from the sloughed off small extra-ovates.

Centrifuged Eggs.

When fertilized eggs were centrifuged with or without previous removal of the fertilization membranes, the normality of development depended upon the centrifugal power employed. In Table II., the effect of centrifugalizing from 35 to 90 revolutions per minute, and from 1 to 15 minutes duration, is shown.[1] When centrifuged at the rate of 35 revolutions per minute for ½ to 7½ minutes (in one experiment for 10 minutes), the subsequent development was normal. When centrifuged 60 or more revolutions a minute for even 1 minute considerable numbers of eggs developed but very atypically. When centrifuged for longer periods at this rate, the entire culture was rendered abnormal. At 90 revolutions a minute the embryos were nearly all atypic even though centrifuged ½ to 1 minute. Beyond this rate, or for longer periods of time, the eggs were either killed outright or broken into small fragments, only some of which developed into the atypic blastulæ or atypic gastrulæ already mentioned. These facts are shown in tabular form in Table II.

Table II.

Fertilized eggs were centrifuged at different rates and for varying periods of time. Within well-defined limits of speed and time the embryos and larvæ were quite normal.

No.	Experiment.		Result.
10*e*	Centrifuged and shaken for 24 minutes.		Few alive. Developed to young plutei only.
20–1	Centrifuged 35 × per min. for	6 min.	Developed to gastrulæ only.
		8	Developed to gastrulæ only.
		10	Developed to gastrulæ only.
		12½	Developed to gastrulæ only.
		15	Developed to blastulæ only. Irregular.
25*c*	Centrifuged 35 × per min. for	1	Developed normally.
		3	Developed normally.
		5	Developed normally.
		8	Developed irregularly.

---

[1] The arms of the centrifuge measured 150 mm. each.

TABLE II.—*Continued.*

No.	Experiment.		Result.
33	Centrifuged 35 × per min. for	5	Developed normally. Some irreg.
		7½	Developed normally. Some irreg.
		10	Developed normally. More irreg.
38	Centrifuged 35 × per min. for	1	Developed normally. More irreg.
		2	Developed normally.
		2	Developed normally.
		3	Developed normally.
		5	Developed normally. More irreg.
		5½	Developed normally. More irreg.
		6	Developed normally. More irreg.
		7	Developed normally. More irreg.
27*c*	Centrifuged 35 × per min. for	6	Developed normally. More irreg.
20–2	Centrifuged 60 × per min. for	6	Developed to young plutei only. Many irreg.
		8	Developed same.
		10	Developed same.
		13	Developed irregularly.
25–10	Centrifuged 60 × per min. for	½	Developed normally.
		1	Developed increasingly irreg.
		2	Developed increasingly irreg.
		4	Developed increasingly irreg.
		5	Developed increasingly irreg.
38*b*	Centrifuged 60 × per min. for	2	Developed to gastrula. Most dead.
18	Centrifuged 60 × per min. for	1	Developed normally. Some irreg.
		2	Developed normally. Some irreg.
		4	Developed normally. Some irreg.
		6	Developed normally. Many irreg.
		8	Developed normally. Most irreg.
		10	Developed normally. Most irreg.
25–20	Centrifuged 90 × per min. for	½	Developed young plutei only.
		1	Developed increasingly irreg.
		2	Developed increasingly irreg.
		3	Developed increasingly irreg.
		4	Developed increasingly irreg.
		5	Developed increasingly irreg.

In an effort to agglutinate the eggs, they were centrifuged not in urine tubes but in finely drawn glass capillaries, plugged at one end with paraffin, and the eggs liberated a few minutes to 24 hours after centrifuging. This method was unsatisfactory, first, because development ceased altogether within the capillaries, and when liberated, after four or more hours, development was atypical. When liberated, after 18 or more hours in the capillaries, they did not develop at all, Table III. Secondly, in removing the eggs, it was necessary to exert considerable force, enough to separate the eggs from one another. The difficulty was overcome by using narrow bore tubes whose inside diameter was about 3 mm. Eggs centrifuged in these tubes within the centrifugal limits already mentioned, gave rise to normal embryos and larvæ many of which were agglutinated or fused.

TABLE III.

The effect of centrifuging eggs in capillary tubes, after they have been fertilized, and had their membranes removed.

No.	Experiment.	Result.
30–2	Not centrifuged.	Kept in capillary ½ hr. Same as control.
3	Not centrifuged.	Kept in capillary 1 hr. Same as control.
4	Not centrifuged.	Kept in capillary 4½ hrs. Same as control. Clusters present.
5	Not centrifuged.	Kept in capillary 18 hrs. Most dead.
25–30	Centrifuged 30 revol. a min. for 1 min.	15 min. Norm. Clusters present.
31	Centrifuged 30 revol. a min. for 1 min.	30 min. Norm. Clusters present.
32	Centrifuged 30 revol. a min. for 1 min.	60 min. Norm. and irregularities.
33	Centrifuged 30 revol. a min. for 1 min.	90 min. Norm. and irregularities.
40	Centrifuged 60 revol. a min. for 1 min.	15 min. Norm. and irregularities.
41	Centrifuged 60 revol. a min. for 1 min.	30 min. Norm. and irregularities.
42	Centrifuged 60 revol. a min. for 1 min.	60 min. Norm. and irregularities.
43	Centrifuged 60 revol. a min. for 1 min.	90 min. Norm. and irregularities.
50	Centrifuged 90 revol. a min. for 1 min.	15 min. Norm. and irregularities.
51	Centrifuged 90 revol. a min. for 1 min.	30 min. Norm. and irregularities.
52	Centrifuged 90 revol. a min. for 1 min.	60 min. Nearly all dead.
53	Centrifuged 90 revol. a min. for 1 min.	90 min. Nearly all dead.
18–29	Not centrifuged.	Kept in capillary 1 hr. Norm.
30	Not centrifuged.	Kept in capillary 2½ hrs. Norm.
31	Not centrifuged.	Kept in capillary 3 hrs. Plutei irreg.
32	Not centrifuged.	Kept in capillary 5 hrs. Few norm. plutei.
33	Not centrifuged.	Kept in capillary 7 hrs. Only gastrula.
34	Not centrifuged.	Kept in capillary 8 hrs. Died early cleavage.
35	Not centrifuged.	Kept in capillary 19 hrs. Died early cleavage.

CALCIUM-FREE SEA WATER.

When eggs with their membranes removed were placed in calcium-free sea water for half an hour, the blastomeres of the two-cell stage separated from one another either completely, and the resulting larvæ were perfect though small, or incompletely, and twin or fused plutei were produced as described by Loeb, Morgan and Driesch. When the eggs were kept in the solution for one hour, each of the four quarter blastomeres developed, some into dwarf plutei, others never beyond the gastrula stage. With increasing exposure to the calcium-free sea water, the increasing diminution in the size of the blastomeres, and the probable increasing segregation of formative stuffs, plutei were no longer formed, and abnormal embryos were correspondingly more numerous, most of which died early.

Solutions made with tap water (Woods Hole, Mass.) were highly injurious, for few eggs developed into normal larvæ. With distilled water practically all were normal, provided the eggs were not left in the solution longer than about thirty minutes as shown in Table IV.

Table IV.

The effect of calcium-free sea water upon the development of fertilized eggs.

No.		Experiment.		Result.
26*b*	10	Left in solution.	15 min.	Dead. Accident.
	11		30	Same as controls and small plutei.
	12		45	More small plutei and non-developing blastulæ.
	13		60	Only small plutei. Very many irreg.
	14		90	Few small plutei. Most irreg. on bottom, dead.
	15		120	Few small plutei. Most irreg. on bottom, dead.
		Membranes removed.		
	1	Left in solution.	15	Norm. and small plutei.
	2		30	Dead. Accident.
	3		65	Norm. and small plutei.
	4		60	Dead. Accident.
	5		90	Plutei and irregular clusters.
	6		105	Some plutei, most irregular, clusters.
		Distilled Water.		
18	24	Left in solution.	½ hr.	Norm. and small plutei.
	25		1	Small plutei and irregular plutei.
	26		2½	Few small plutei. Most irregular.
	27		6	Most irreg. Nearly all dead.
	28		7	Most irreg. Nearly all dead.
		Tap water.		
	17	Left in solution.	½ hr.	Few norm. plutei.
	18		1	Irregular plutei and irregular gastrulæ.
	19		2½	Only blastulæ irreg.
	20		6	Only blastulæ irreg.
	21		7	Only blastulæ irreg. Few alive.

THE EFFECT OF NaOH.

The gelatinization of the egg by the addition of NaOH and other alkalines to sea water aids materially in the agglutination. When 25 drops or more of 0.5 per cent. NaOH solution was added to 200 c.c. of sea water, the gelatinization was so great, that after centrifuging, the eggs were distorted almost beyond recognition. Figs. 6 and 7, on page 75, give an inadequate idea of the degree of distortion. Less NaOH, namely, 4 to 10 drops in the same amount of sea water, gave rise to no abnormalities.

From the foregoing it is clear that within the limits specified, the various steps found necessary in the production of agglutinated eggs, either separately or all together, did not materially effect development, except for the small fragments which gave rise to atypic blastulæ and gastrulæ, already mentioned. Table V. gives a list of experiments in which numerous agglutinated and fused embryos were observed.

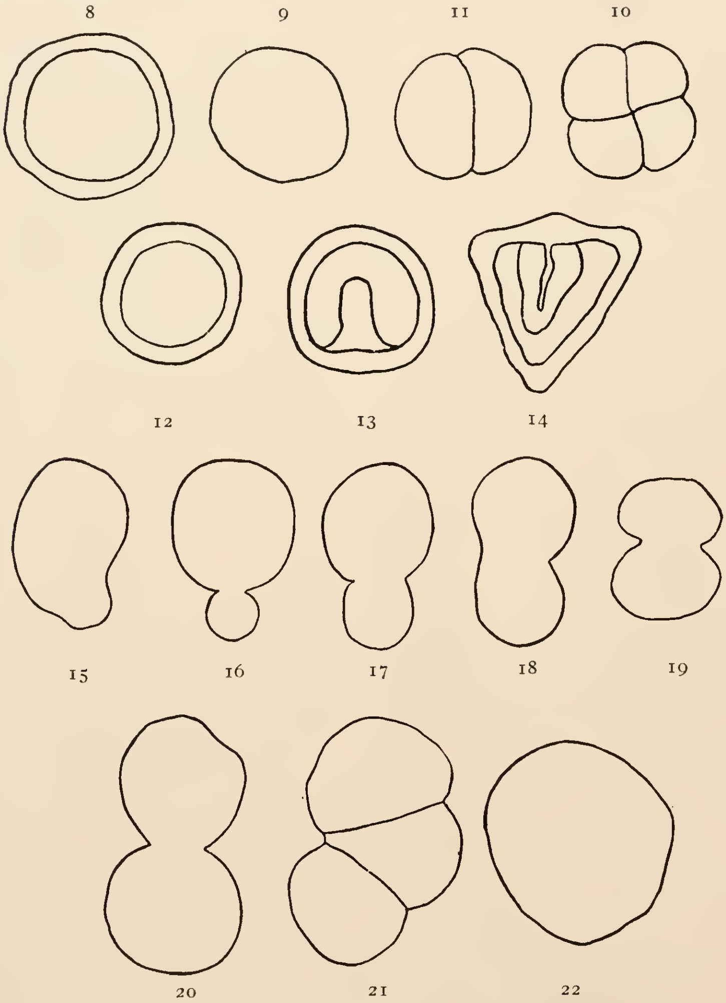
TABLE V.

This table gives a list of experiments in which agglutinated and fused eggs were produced.

Exp. No.		Method Used.	
20.3		Fertilized. shaken, calcium-free solution, NaOH centrifuged 35×,	5 min.
	3*b*2	Fertilized, shaken, calcium-free solution, NaOH centrifuged 35×,	7½
	3*b*4	Fertilized, shaken, calcium-free solution, NaOH centrifuged 35×,	12½
25	*c*5	Fertilized, shaken, calcium-free solution, NaOH centrifuged 30×,	6
	*c*6	Fertilized, shaken, calcium-free solution, NaOH centrifuged 30×,	8
27	*b*	Fertilized, shaken, ... NaOH centrifuged 35×,	6
	ce	Fertilized, shaken, calcium-free solution, NaOH centrifuged 35×,	5
33	*d*	Fertilized, shaken, calcium-free solution, NaOH centrifuged 30×,	10
34	*d*	Fertilized, shaken, calcium-free solution, NaOH centrifuged 30×,	4½
37	*b*3	Fertilized, shaken, calcium-free solution, NaOH centrifuged 35×,	3
		Fertilized, shaken, calcium-free solution, NaOH centrifuged 35×,	5
		Fertilized, shaken, calcium-free solution, NaOH centrifuged 35×,	7
	*d*1	Fertilized, shaken, calcium-free solution, NaOH centrifuged 60×,	2
	*d*2	Fertilized, shaken, calcium-free solution, NaOH centrifuged 35×,	2
	*d*3	Fertilized, shaken, calcium-free solution, NaOH centrifuged	3
	*d*4	Fertilized, shaken, calcium-free solution, NaOH centrifuged	5
	*d*5	Fertilized, shaken, calcium-free solution, NaOH centrifuged	7½
	*e*2	Fertilized, shaken, calcium-free solution, NaOH centrifuged	2
38	*b*3	Fertilized, shaken, calcium-free solution, NaOH centrifuged	2
	*d*7	Fertilized, shaken, calcium-free solution, NaOH centrifuged	5
	*d*8	Fertilized, shaken, calcium-free solution, NaOH centrifuged	7
Etc.			

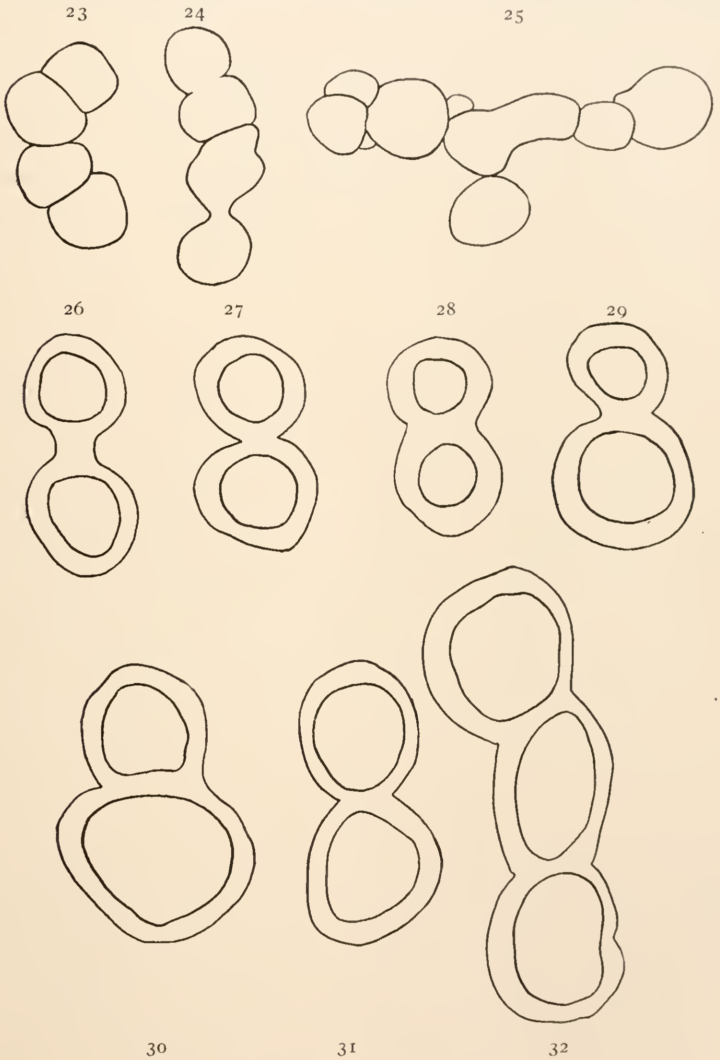
THE EARLY DEVELOPMENT OF AGGLUTINATED EGGS.

To serve as a basis of comparison, a few typical stages in the development of the normal egg are shown in Figs. 8 to 14. These were preserved, magnified and drawn with the camera lucida, to the same scale, as all subsequent ones to be described. In Fig. 8, the egg is enveloped by its fertilization membrane. In Fig. 9 the membrane is not shown. The two and four cell stages



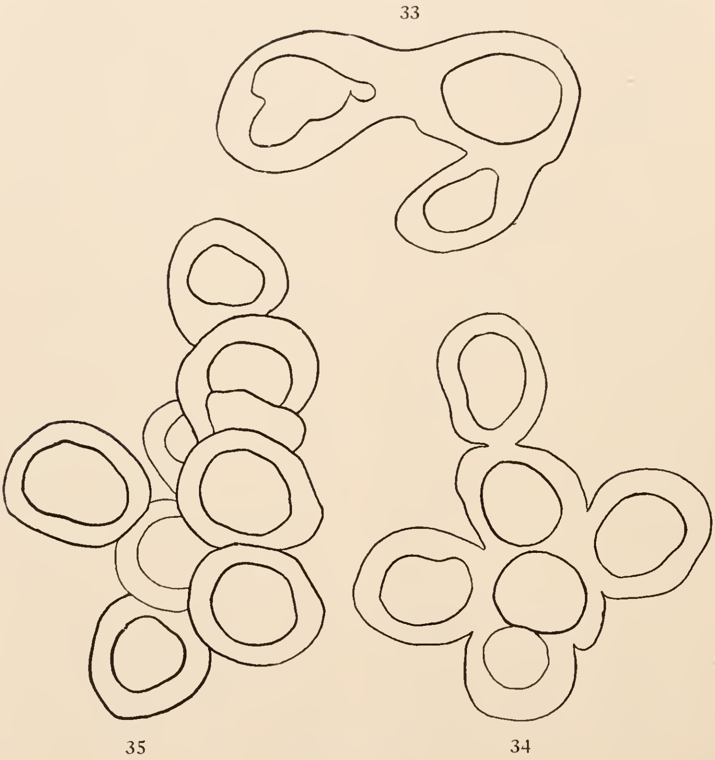
FIGS. 8–22.

are shown in Figs. 10 and 11. The blastula, gastrula, and young pluteus are shown in Figs. 12, 13 and 14. It need hardly be iterated that the eggs and embryos in the control cultures varied within certain limits, as carefully worked out by Tennent ('10); the above figures represent typical or average specimens.



Figs. 23–32.

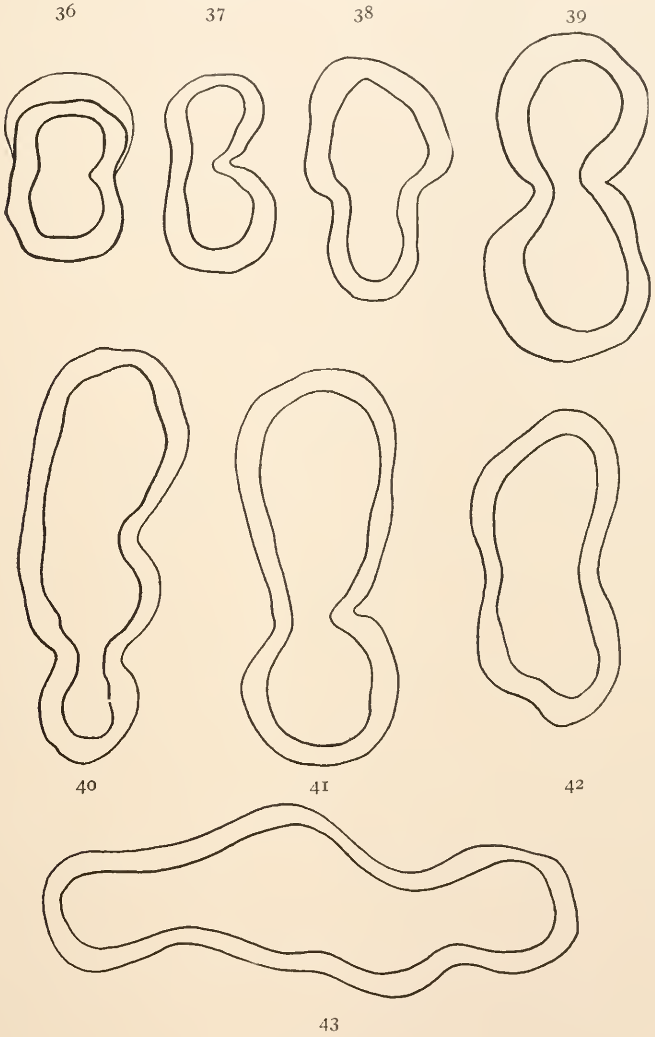
The treated eggs differed from the controls, in being elongated or extra-ovate as seen in Figs. 15, 16 and 17. In Figs. 17, 18 and 19, the two parts of the egg are equal or almost equal. It is sometimes difficult to distinguish between an egg with its equal-sized extra-ovate, from one whose two blastomeres have been partially separated. In either case the volume of the whole egg appears to be increased, sometimes suggesting an agglutination of two whole eggs, as in Figs. 18 and 19. In Fig. 20 two eggs have unmistakably agglutinated. Sometimes three eggs are compressed together as in Fig. 21, or *fused completely* as in Fig. 22.



Figs. 33–35.

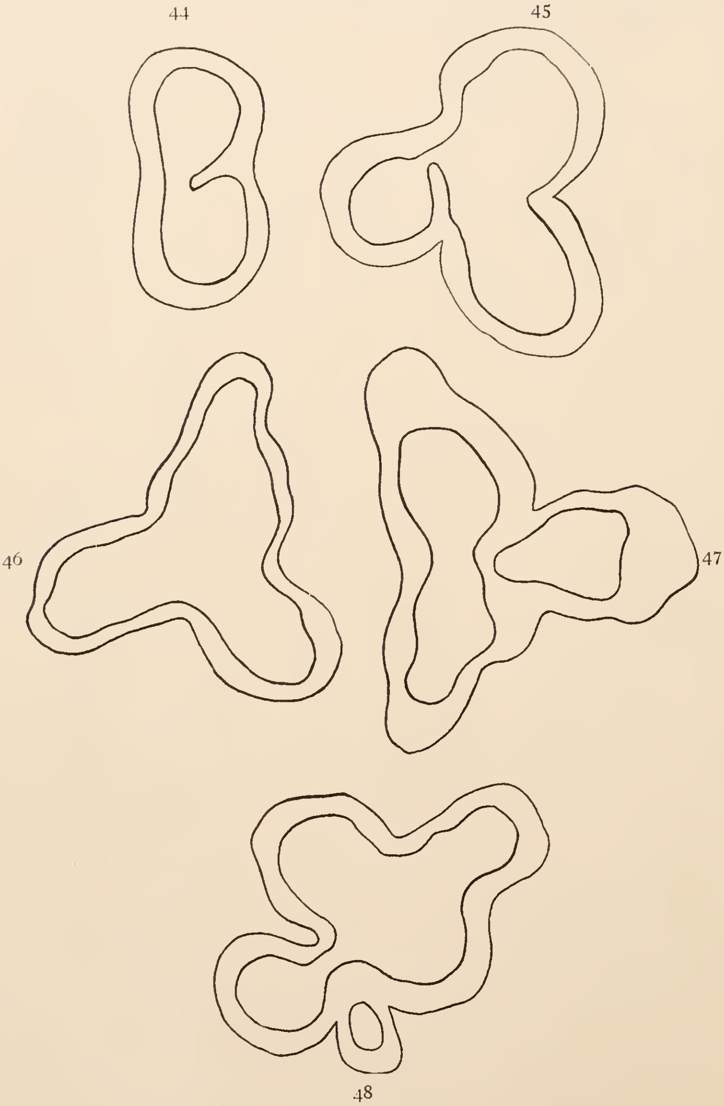
Since the eggs or blastomeres may be agglutinated at any point of the surface and since the polarity of the egg is unaffected, the cleavage planes may occupy any angle with respect to one another, see Figs. 23, 24 and 25. Unless considerably distorted as in Figs. 6 and 7, each blastomere divided at an angle determined by the angle of agglutination or separation.

The blastulæ are particularly interesting for they make it possible to determine definitely whether the component members of a cluster are merely agglutinated or fused. Figs. 26, 27 and 28 represent half blastomeres in varying degrees of approximation or separation, with their blastocœles entirely separate. Figs. 29 and 30 represent an agglutination of a half and a whole egg,



Figs. 36–43.

Fig. 31 an agglutination of two eggs. Three agglutinated eggs are shown in Fig. 32 and two and a half eggs in Fig. 33. Clusters



FIGS. 44–48.

of seven and nine eggs and blastomeres are shown in Figs. 34 and 35.

In a large number of instances a true fusion occurred during the blastula stage, as recognized by the continuous ectoderm and common blastocœle. In Fig. 36 a blastula is shown with a part extruded beyond the fertilization membrane. In Fig. 37 the blastula is pinched together near the middle. A whole and a half egg have been fused in the formation of the single blastula shown in Fig. 38. At least two eggs have united in Fig. 39. In Fig. 40 an egg a half egg and a quarter egg have so fused. Figs. 41, 42 and 43 represent other fusions of two or more eggs into single giant blastulæ. Figs. 44 and 45 are interesting because they suggest how a common blastocœle may be formed by the breaking down of the separating wall.

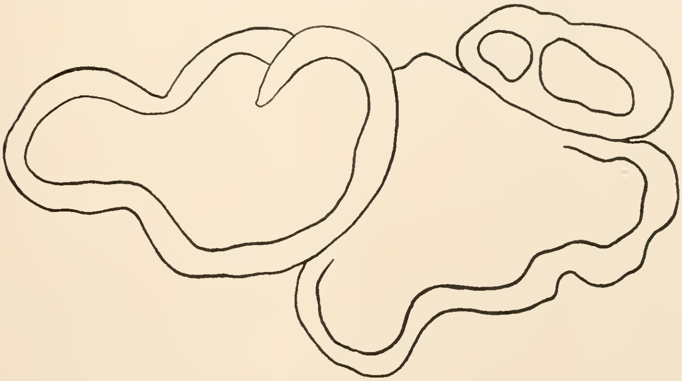
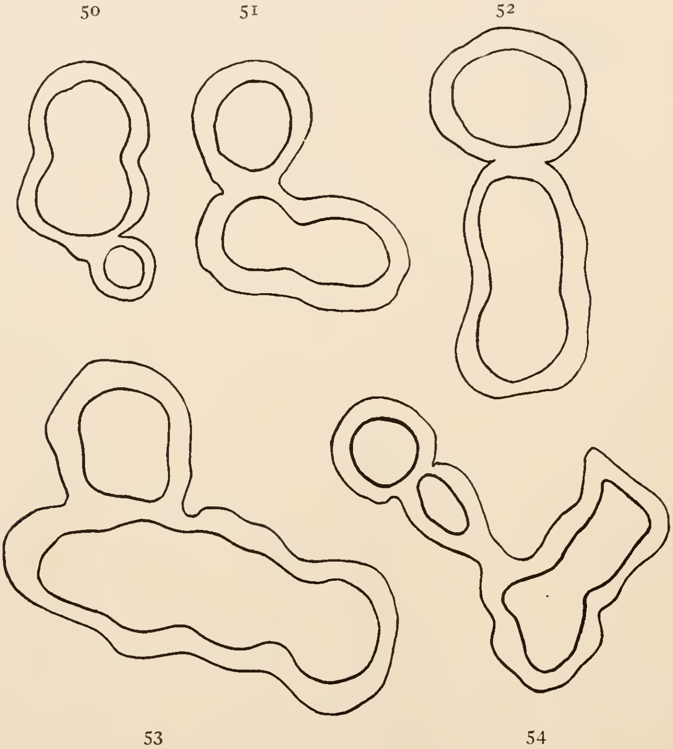


FIG. 49.

The clusters were not necessarily linear. Triangular groups like Figs. 45, 46 and 47 were not uncommon. Other clusters were quite irregular as in Figs. 48 and 49. A large cluster like the one shown in Fig. 49 was more frequently composed of agglutinated eggs or a complex of fused and agglutinated eggs; smaller clusters also included agglutinated and fused members shown in Figs. 50, 51, 52, 53, 54, 47 and 48.

Fused blastulæ tended to lose their individual identity by the continuity of their common layer of cells, by the disappearance of their inner separating walls and by the closer approximation of the separate blastulæ. These processes continued until a spherical or almost spherical giant blastula was produced, in which it was difficult or impossible to distinguish the component members, as in Figs. 55, 56 and 57.

The living clusters of embryos were easily distinguished from the normal ones. The latter swam with a characteristic uniformly rotary motion at or near the surface of the water. Agglutinated or fused blastulæ swam at or near the bottom swaying irregularly and moving much more slowly. I have counted as many as fourteen full-sized blastulæ, of which Fig. 58 represents a large group of this kind. These are short lived, as already mentioned, and only the smaller clusters continue their development.

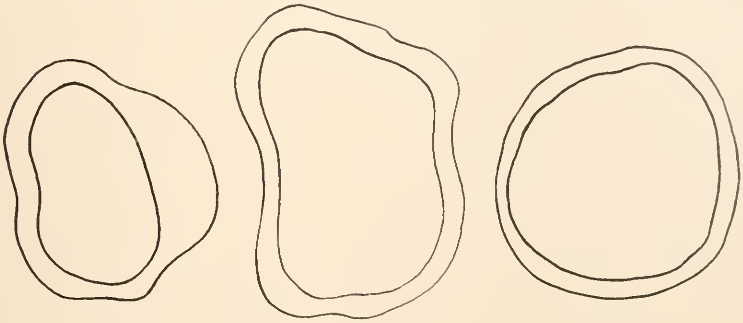


FIGS. 50–54.

THE DEVELOPMENT OF AGGLUTINATED AND FUSED GASTRULÆ.

The differentiation of the archenteron definitely established the axes of the component members. Inasmuch as half eggs developed in precisely the same manner as whole eggs, the following statements apply to both.

The axes of the archenteron in the different gastrulæ of a cluster were found in every possible angle with respect to one another, suggesting that the polarity of the egg was unaffected by the proximity of the other. Two gastrulæ with their oral ends together, and their archentera in the same axis are shown in Fig. 59, and similar gastrulæ with their archentera almost in the same axis are shown in Fig. 60. The apex or aboral end of one gastrula may be attached to the side of the other and their archentera about 90° apart as in Figs. 61 and 64, or almost parallel in Fig. 62. The two gastrulæ may be attached at or near their aboral ends, Fig. 63. In Fig. 65 the oral end of one gastrula is attached to the aboral end of the second, and the archentera lie in an almost continuous line. In Fig. 66 the archentera lie in the same axis, but the gastrulæ are attached by their aboral ends. Many other examples could be cited. They clearly point to the conclusion that at this stage of development the polarity of the gastrulæ need not be changed by the proximity of other.



56 55 57

FIGS. 55–57.

FUSED GASTRULÆ.

The same conclusion obtains with reference to fused gastrulæ. In Fig. 67, the blastopores are at opposite ends, namely 180° apart and the archentera have grown toward each other in the same axis. In Fig. 68, the blastopores are about 45 degrees apart and the archentera have crossed each other at this angle. Perhaps these two instances suffice as examples of this kind of independent development of the archentera. In other instances however the archentera met and fused.

In Fig. 69, the archentera have approximated but not yet fused into one continuous gut. In Fig. 70 the two have unmistakably united into a single continuous gut with no trace of blastopores.

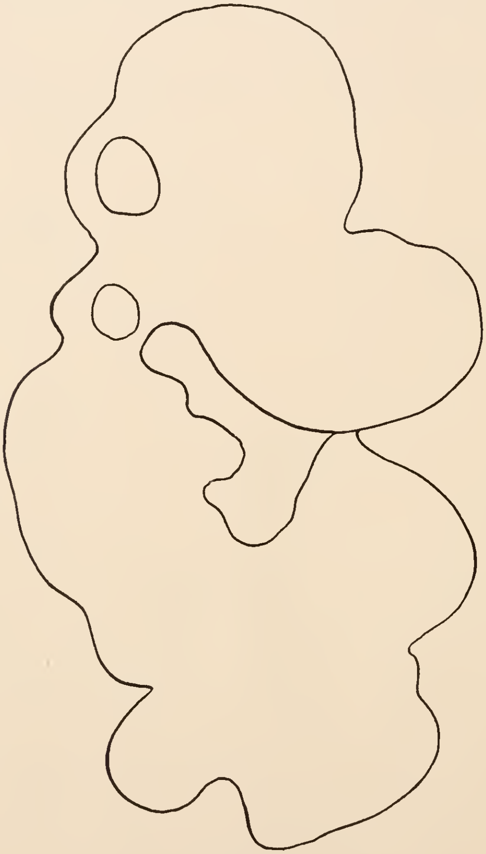
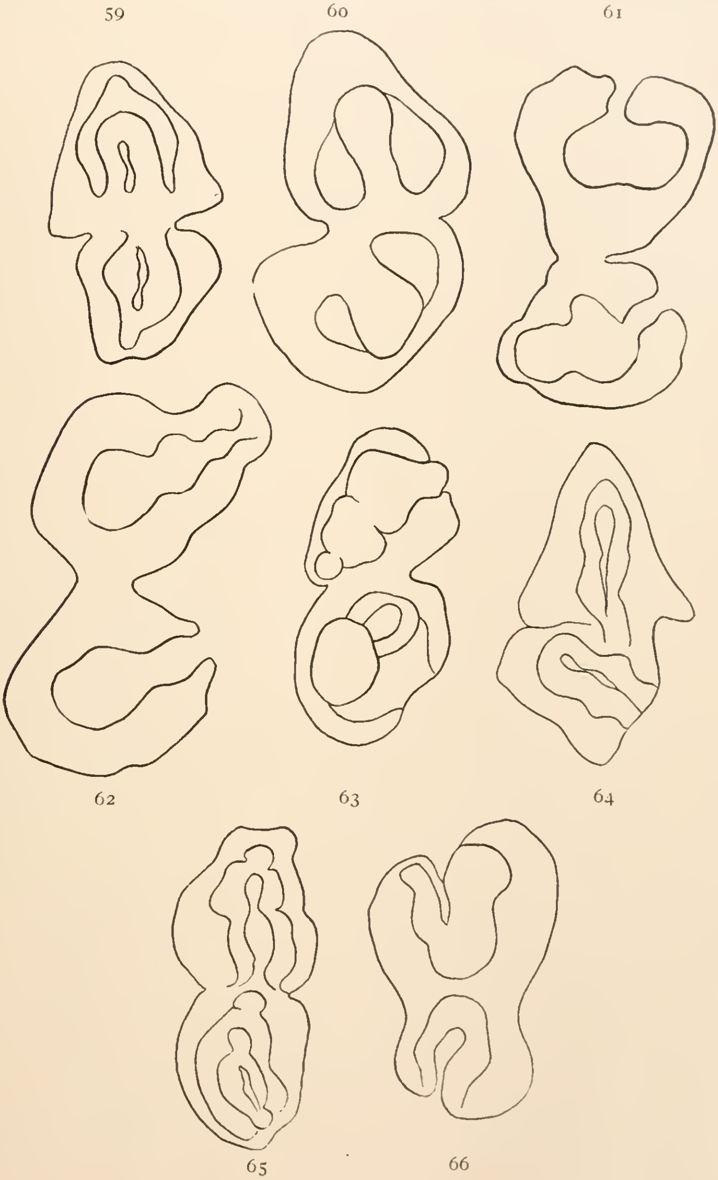


FIG. 58.

A similar gastrula drawn from life and drawn at greater magnification than the rest is shown in Fig. 71. In a cluster of four gastrulæ two have separate guts, two have united ones, Fig. 72.

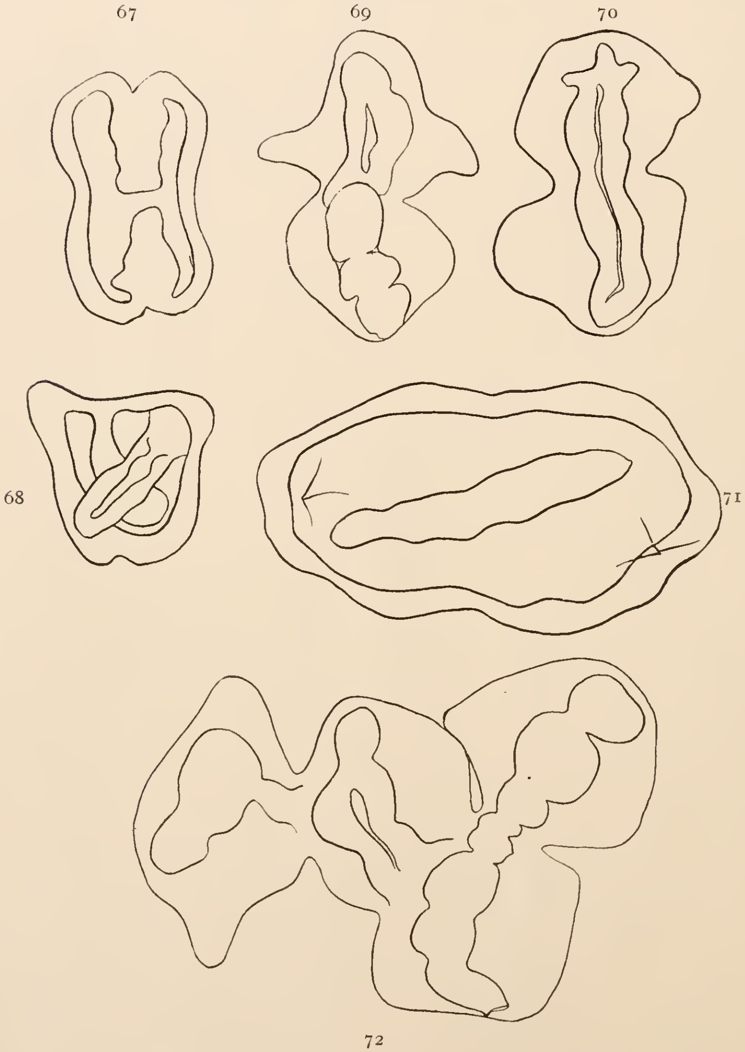
Instead of fusing end to end, the archentera may unite near their middle as in Fig. 73, or the blastopores with or without parts of the archentera may be united as in Figs. 74, 75 and 76. If one were to suppose the fusion of two archentera side to side, as suggested by the preceding figure, one would anticipate the single giant archenteron shown in Figs. 77 and 78.

There were in the different cultures two other kinds of fusion that deserve brief mention. In the one, a gastrula developed in only one of the pair of eggs or blastomeres as shown in Figs.



Figs. 59–66.

79 and 80. The archenteron in such fusions may grow beyond the boundaries of the blastomere or egg into the blastocœle of its neighbor as seen in Fig. 81. In the second group the archentera

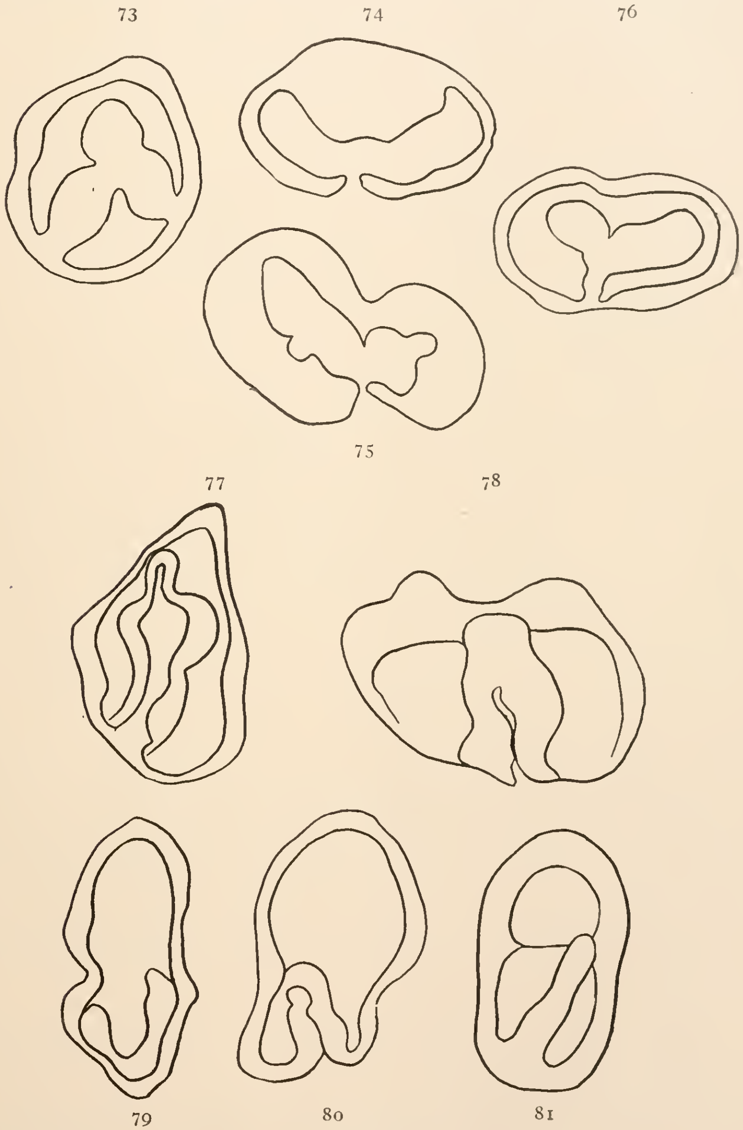


Figs. 66–72.

are usually atypic either detached as in Fig. 82 or independent, Figs. 83 and 84.

THE DEVELOPMENT OF AGGLUTINATED AND FUSED PLUTEI.

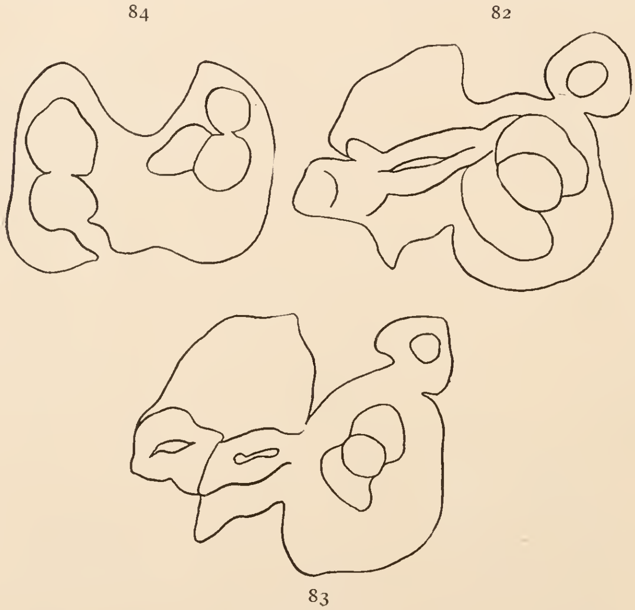
It will be recalled that the agglutinated embryos tended to separate and that this tendency increased during development. When the pluteus stage was reached, very few remained aggluti-



FIGS. 73–81.

nated, and these were in nearly every instance, clusters of two. The separation of the embryos of *Arbacia* occurred in exactly the same manner as described by Driesch.

These permanently agglutinated plutei may be attached to one another at any angle as in Figs. 85, 86 and 87. In Fig. 85 the plutei are attached by their oral ends; in Fig. 86, the oral end of one is attached to the side of the other, and in Fig. 87 a group of five plutei are agglutinated at various angles. As in the previous stages of development one finds here also, that the agglutinated partners may develop at different rates, the one a pluteus, the other a blastula as Fig. 88, or a pluteus and a gastrula, Fig. 89.



FIGS. 82–84.

Driesch described the following types of plutei from his cultures:

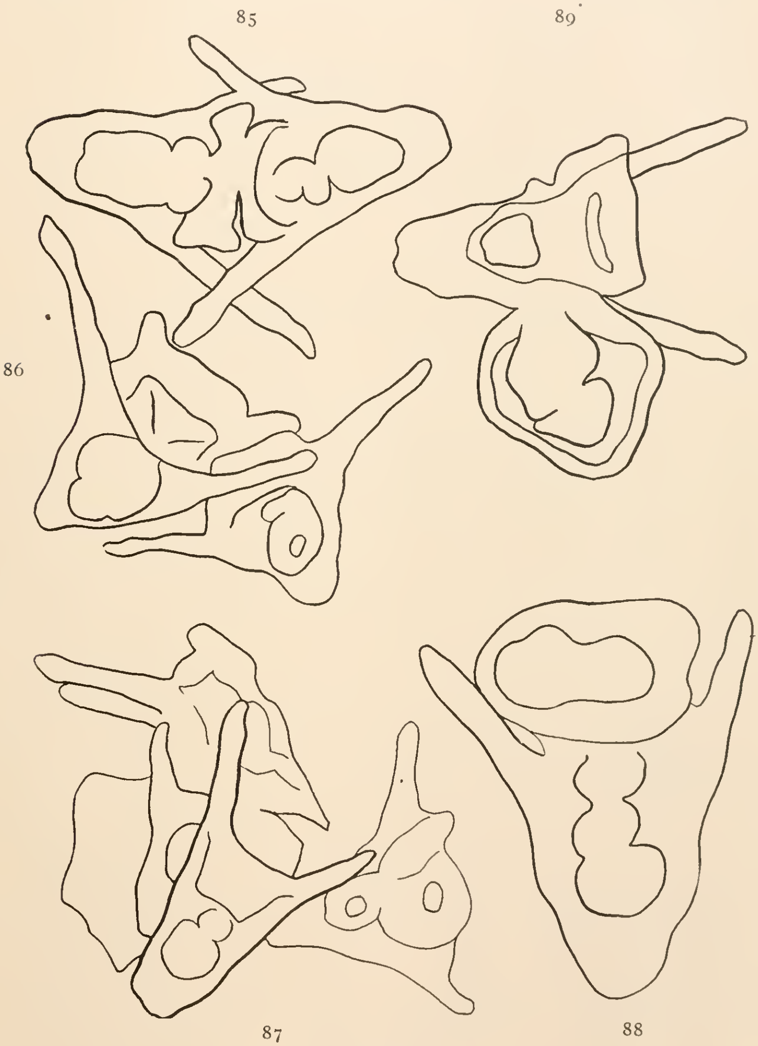
(*a*) True twins, *i. e.*, agglutinated plutei.

(*b*) Twins with a common blastocœle, *i. e.*, body wall partially fused, internal organs double.

(*c*) Twins with a reciprocal influence on growth, a true fusion with an enlargement of the body.

(*d*) Fusion with partially double archenteron.
(*e*) True fusion with a single set of organs.
(*f*) Single body with a second parasitic archenteron.

Some of these types he obtained from *Echinus microtuberculatus* only, types *a*, *b*, *c*, *d* and *e*; others from *Sphærichinus granularis*, types *e* and *f*; and some from both of these echinoderms, type *e*.



FIGS. 85–89.

Since Driesch has so clearly and accurately described these types, there is no need to enter into detail except to mention that with *Arbacia punctulata*, I obtained all of them, namely:

Type *a*, or true twins, Figs. 85, 86 and 87.

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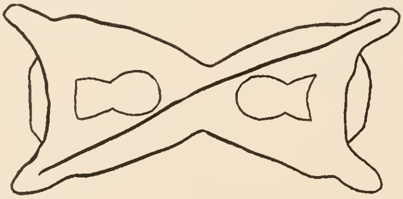

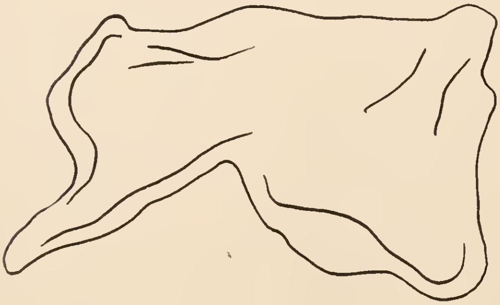

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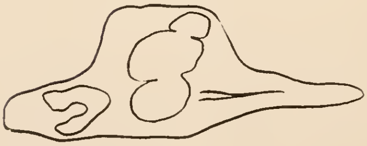

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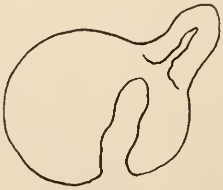

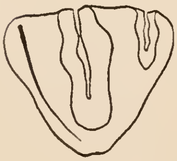

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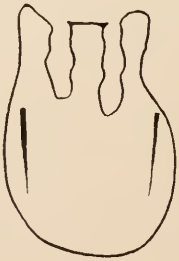

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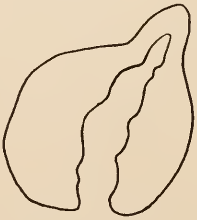

96

Figs. 90–96.

Type *b*, pair of plutei with a common blastocœle, Fig. 90, drawn from life at greater magnification.

Type *c*, fusion of two, but with double set of organs, very few examples, Figs. 94 and 82.

Type *d*, fusion of two with partially double archentera, Figs. 73, 74 and 75.

Type *e*, complete fusion with but a single set of organs, the pluteus of Figs. 88 and 78.

Type *f*, single body with a second parasitic archenteron, Figs. 84, 92 and 93.

The Development of Individual Clusters.

In an effort to follow the developmental and regulative processes, during agglutination and fusion, each cluster was isolated and examined at periodic intervals. The very large groups separated into their component members, as already pointed out, each developing into a normal larva or the inner ones disintegrated bringing about the disintegration of the entire cluster. The very small groups usually remained intact. Sketches and memoranda made during the development of these small clusters clearly showed that the types of agglutinated and fused eggs, embryos and larvæ described in the previous section, were not the artificial results of preservation, but represented various serial steps in the development and regulation of such clusters. To describe these isolated groups would be to repeat the descriptions of preserved material given in the preceding section.

This statement applies with equal force to fused clusters, though not all types were observed in the isolated clusters. The following are some of the true fusions observed:

Two eggs fused into a single body with two independent archentera equal in size, Fig. 94,[1] or unequal, Fig. 93.

Two eggs fused into one body with two archentera attached end to end.

Three eggs fused into one body with three independent archentera.

Two fused eggs and one egg fused to a blastomere developed independent archentera, Fig. 95, which subsequently came in contact and fused into one very long archenteron, Fig. 94.

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[1] Figs. 93 to 95 are drawn from hand sketches.

Two eggs fused into one pluteus with independent archentera, one very much larger than the other.

Two eggs fused completely into one pluteus body with one set of organs.

Other clusters showed distinct retrogressive and involutional changes of which the following are a few examples:

Four blastulæ were agglutinated in a row. The end ones enlarged, the inner ones became small. One of these small blastulæ developed an archenteron which later shrank and disappeared completely. Certain other changes occurred which altered the character of the group so that ultimately three minute blastulæ were crowded together at one point on the periphery of a large blastula.

Two large fused blastulæ were attached to a third blastula very much smaller. The three fused into one body with three independent archentera. The next day the small archenteron disappeared, skeletal rods differentiated on one side of the body constituting a fusion of a pluteus and a gastrula. Two days later the parts had fused more completely, into a single body with a single normal-sized archenteron and a single skeleton.

Two eggs developed into fused gastrulæ and later into fused plutei, attached by their oral surfaces. One of the plutei decreased in size while the other increased correspondingly, the total linear dimensions remaining constant. The smaller pluteus ultimately became less than one quarter its original size.

SUMMARY.

Prior to this work, no one had succeeded in fusing at will the eggs of any animal found on this side of the Atlantic. By a modification of the Herbst-Driesch method, described in the text, it was possible to agglutinate and to fuse relatively large numbers, namely 10 to 40 per cent. of the sea urchin *Arbacia punctulata*.

Such clusters whether studied in mass cultures or in isolated groups, developed into all the types of larvæ described by Driesch for *Echinus microtuberculatus* and *Sphærechinus granularis*, namely:

(*a*) True twins.

(*b*) Twins with a common blastocœle.
(*c*) Twins with a reciprocal influence on their growth.
(*d*) Fusion with partially double archentera.
(*e*) True fusion with a single set of organs.
(*f*) Single body with a second parasitic archenteron.

Since little attention has been given to the early development of such agglutinations and fusions, the text emphasizes the earlier stages summarized as follows:

Clusters of 2 to 20 eggs and blastomeres were successfully agglutinated. The large clusters nearly always disappeared either by the separation of the outer members or by the death and disintegration of the inner ones. Small clusters of 2, 3 or 4 eggs or blastomeres survived and either remained agglutinated or were fused.

The eggs were agglutinated either at the egg stage or during the formation of the blastula. In clusters which remained agglutinated and did not fuse, the members developed independently, *i. e.*, the polarity was not affected by the proximity of the other, and the rate of development was not necessarily different in the individual members of a cluster.

Fusion occurred infrequently at the egg stage, but more commonly took place during the blastula or later stages. Such fusion involved either the body wall, one or more of the internal organs or all of these.

Fusion was frequently determined by the degree of compression of the component eggs or blastulæ, as well as the position and angle of attachment.

Embryos fused end to end frequently developed a single archenteron, about twice the normal size, either with or without blastopores.

Embryos fused with their axes 90 degrees apart, frequently fused in such a manner that their archentera united at the point of contact.

Embryos fused with their axes parallel and close together, frequently fused in such a manner that the two archentera fused into one, along their whole length.

The study of individual clusters served to show that these types of agglutination and fusion represented regulative and sometimes involutional stages in the development of these clusters.

The evidence supports the view that not only may an egg give rise to several perfect larvæ, but that several eggs may be united so as to constitute a single larva, with or without traces of its duplicate nature.

In conclusion I wish to thank Prof. F. R. Lillie for placing the facilities of the Marine Biological Laboratory at Woods Hole, Mass., at my disposal.

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ON THE HABITS OF THE CRUSTACEANS FOUND IN CHÆTOPTERUS TUBES AT WOODS HOLE, MASSACHUSETTS.

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Among the numerous species of crustaceans which live as commensals, three interesting representatives occur in the leathery tubes of the worm *Chætopterus variopedatus* Renier et Claparède, at Woods Hole. The tubes of this annelid are U-shaped and taper toward the round opening at either end (Fig. 7). The tips of the tube may be seen protruding above the mud in open shallow water where eel-grass grows. Enders ('05, '09) has published two papers describing the habits of *Chætopterus* and its commensals as he observed them at Beaufort, North Carolina. Van Beneden ('76, p. 20) also mentions a worm, which is doubtless the same, as associated with crabs on the coast of Brazil. While occupying a room in the Marine Biological Laboratory during the past summer I was able to verify Ender's work and to add some new observations. My thanks are due to the staff of the laboratory for their courtesy, particularly to Mr. George Gray.

Enders ('05) took four species of crustaceans (*Polyonyx macrocheles* (Gibbes), *Pinnixa chætopterana* Stimpson, *Pinnotheres maculatus* Say, *Menippe* sp.?) from tubes at Beaufort. All of these crustaceans except *Menippe* were taken at Woods Hole. Usually a male and a female of the same species were found together in each tube, but several solitary individuals were captured, and once two *Pinnixæ* of each sex were found in a single tube. The results of the collections at the two localities are compared in Table I. The table shows that *Polyonyx* is the most abundant commensal at Beaufort, whereas *Pinnixa* is the most frequent at Woods Hole. Mr. George Gray, who has collected *Chætopterus* for several years at the latter place, affirms that he had never observed *Polyonyx* until about 1909, and that

SHOWING THE NUMBER OF ANIMALS FOUND IN *Chætopterus* TUBES.

Locality.	*Polyonyx macrocheles.*		*Pinnixa chætopterana.*		*Pinnotheres maculatus.*		*Menippe* sp.?	*Nereis* sp.?	*Pinnixa* bearing *Hippuraria elongata.*		Number of *Chætopterus* Tubes, with or without Commensals.		
	♂	♀	♂	♀	♂	♀			♂	♀	Present.	Lacking.	Total.
Enders at Beaufort, N. C.	71	73	15	15	2	2	1	2	–	–	89	10	99
At Woods Hole	14	19	57	61	2	0	0	–	4	7	80	9	89

it has been increasing in numbers since then. The size of the crustaceans collected at Woods Hole was about the same as those measured by Enders ('05) at Beaufort. The maximum width of the carapace recorded for *Polyonyx* was: female, 12.5 mm.; male, 9 mm.; *Pinnixa:* female, 12.5 mm.; male, 13 mm. The proportion of tubes containing commensals was about the same at the two places.

Pinnotheres was not studied on account of its infrequent occurrence, but the behavior of *Pinnixa* and *Polyonyx* was observed in some detail. Both the latter crustaceans are thigmotropic. When placed in a dish they usually stayed close in the angles at the edge, or crawled under any objects that were present. *Pinnixæ* often clung to each other or piled up in groups. If supplied with glass tubes of suitable size, they crawled into them and there remained indefinitely.

In order to test the behavior of the commensals toward *Chætopterus* tubes an artificial tube was made in which their movements could be observed. A glass tube was bent in the form of a U and the tip of a *Chætopterus* tube slipped over each end where it was tied securely. This composite tubewas placed upright in a rectangular glass jar filled with sand so that the glass tube could be observed through one side of the jar, though only the leathery tube tips projected into the sea water above the sand. The crustaceans were placed on the surface of the sand and their behavior observed. *Pinnixæ* usually walked to one side of the jar where they burrowed into the sand and remained for several minutes; the *Polyonyces* moved quickly to the side of the jar and crouched on the sand. None of the crustaceans moved at once to the projecting tube tips, and sometimes they even came in contact with a tube without reacting. After a time, however, they would begin slowly exploring the surface of the sand, and, if they then came in contact with a tube, usually entered at once, whether a *Chætopterus* was inside or not. Apparently they found the tubes by accident.

In regard to the ability of the commensals to leave the tube Van Beneden ('76, p. 20) says, "On the coast of Brazil, my son found two couples of crabs in the tube of a very long annelid, narrow at the ends, and wide in the middle. The tube was too small at the end to allow them to escape. These crustaceans had, no doubt, penetrated thither before they had attained their full size. Enders ('05, p. 39) also believes that, "Once in, the crabs remain there and are later prevented through their own growth from escaping. . . . When a worm in a tube dies the crabs in the same tube die as a result of the failure of food and properly aerated water." Some tubes may be too small to allow the commensals to pass in and out, but there can be no doubt that they enter and emerge again in some cases, as the two following experiments show.

Experiment 1.—On July 19 three *Pinnixæ*, two males and a female, were placed on the sand in the jar containing the tube previously described, which contained a living *Chætopterus*. Next morning all had entered the tube; the *Chætopterus* was dead. At 2.00 p. m. two of the crabs had come out on the surface of the sand, but one still remained in the tube.

Experiment 2.—After the last experiment the tube was removed, thoroughly cleaned, and replaced in the jar with fresh sand and sea water, but no *Chætopterus* was placed in it. On July 20, at 5.00 p. m., the two largest *Pinnixæ* obtainable (male, 13 mm. wide; female, 12.5 mm.) were placed on the sand; at 9.00 a. m. the next morning the male was in the tube, and at 12.30 p. m. the female had entered. On July 22, at 3.00 p. m., a male (9 mm.) and a female (12.5 mm.) *Polyonyx* were also placed in the jar. The male entered at 3.21 and the female at 3.40 p. m. All four crabs remained within the tube until July 24 at 4.45 p. m., when I observed that the water had become foul;

the female *Polyonyx* had left the tube and was on the surface of the sand. The other three crustaceans stayed within until July 27, at about 8.00 p. m., when the male *Polynyx* emerged. The water in the jar at that time was murky, but did not have a very bad odor. On the morning of July 29 the water had some odor; the *Polyonyces* were dead on top of the sand and the *Pinnixæ* had left the tube but were alive and active.

The crabs in experiment 2 were the largest obtainable and the apertures at the ends of the tube tips were of medium size. They seemed to have little difficulty in passing in or out, though one would not think so at first glance. *Pinnixa* walked in sideways; *Polyonyx* extended its chelæ and one of them preceded the body, the other followed.

Locomotion outside the tubes was first studied in a large flat dish containing sea water. *Pinnixa* did not use its last leg when walking forward, but when going sideways used all the walking legs, except the chelipeds, which were hugged close against the body; it was never seen to move backward. *Polyonyx* usually moved backward when in the open and sideways along the sides of the dish. In the latter situation it always faced toward the center of the dish, or, if in haste, stood with its ventral surface against the side of the dish and walked along on the tips of its chelipeds and the bristles along their outer edges (Fig. 1). If very much hurried an animal sometimes turned on its back and flapped its abdomen, thus swimming slowly. When placed in a vertical glass tube filled with sea water *Polyonyx* kept from falling by bracing the fringe of bristles on the chelipeds and the second and third walking legs forward; the fourth leg serving as a prop behind (Fig. 7). The small fifth leg was not used. Locomotion was always sideways. In a similar situation *Pinnixa* braced with all its walking legs; the first and second were extended forward, the third below, and the fourth and fifth

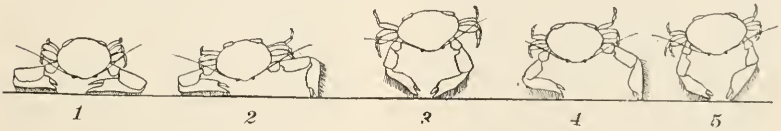


FIG. 1. Diagrams to represent the method of locomotion used by *Polyonyx* when resting on its setal fringe with its body against some object.

backward. The largest and strongest leg, the fourth, served as a sort of hook to grasp any inequality in the wall of the tube, and the smaller fifth leg was often used in much the same way. In a horizontal tube *Pinnixa* moved sideways or stood in a position like that when on a flat surface; *Polyonyx* usually rested on the fringe of bristles along the chelipeds with its head down. *Pinnixa's* most effective locomotor organs are the fourth walking legs: *Polyonyx* uses the chelipeds most.

Pinnixa is an expert burrower, but *Polyonyx* has little ability in that line. When placed in a bowl containing sand and sea water a *Pinnixa* would scratch for a moment with all the walking legs except the chelipeds, then stop moving the legs on one side and quickly burrow sideways by scraping the sand away with those of the other. Usually one would bury itself completely in a minute or two. If *Polyonyx* was placed in the same bowl it did not burrow, in fact was never seen to burrow, though occasionally an individual would wiggle itself down into the sand a little.

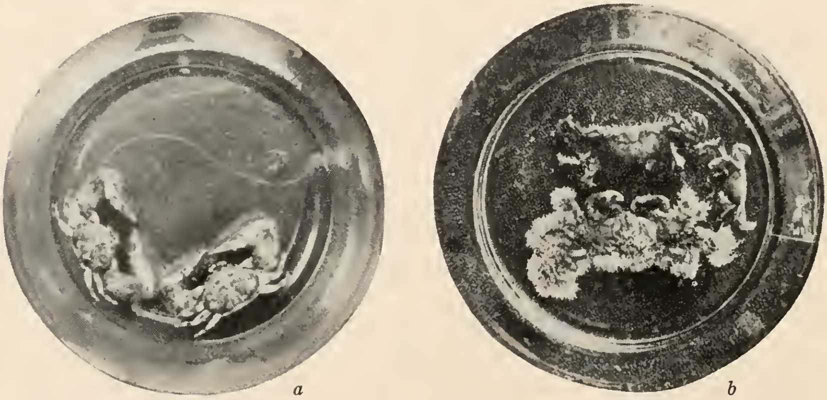


FIG. 2. *Polyonyx* and *Pinnixa* in Syracuse watch glasses. × 4/7. *a, Polyonyx*—male at left, female at right. *b, Pinnixa;* the lower individual is covered by the bryozoan *Hippuraria elongata* Osborn.

Respiration is a matter of importance to an animal that lives in a worm tube imbedded in a muddy flat. Yet *Pinnixa* apparently takes no special precautions to secure well-aerated water and is able to endure a much greater degree of foulness than *Polyonyx*. Its respiratory currents are feeble and inconstant both as to force and direction. In *Polyonyx*, however, such currents are strong and well adapted to life in tubes. When an individual is placed in a dish of water, a current will soon be seen to issue from beneath the anterior end. Sometimes it passes straight forward, but more often moves to the right or left,—as it would have to do if the crustacean were in a tube. The direction of the respiratory current in a particular individual may change frequently in a dish, but in a tube it is usually constant for hours at a time. Furthermore, several individuals in a tube together will adopt the same direction so that the water passes in one end of the tube and out the other. *Polyonyx's* antennules assume a peculiar position in relation to the respiratory current. Both are bent against the current and take such a position that the smooth ramus (Fig. 5*a*) meets it first; and if the current is changed, the position of the antennules is always

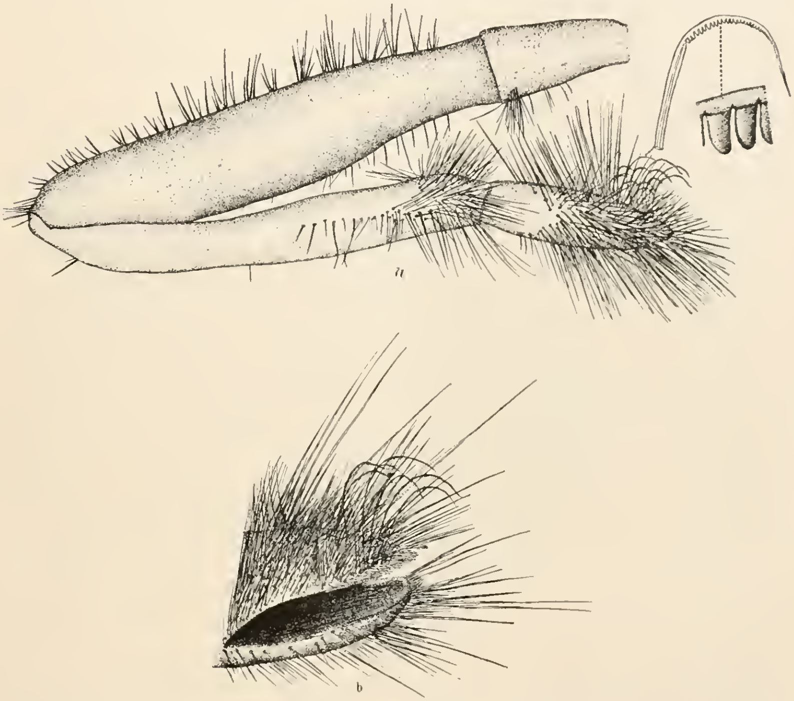


FIG. 3. Cleaning leg of *Polyonyx*. *a*, entire leg with details of pectinate setæ; *b*, chelate tip of leg.

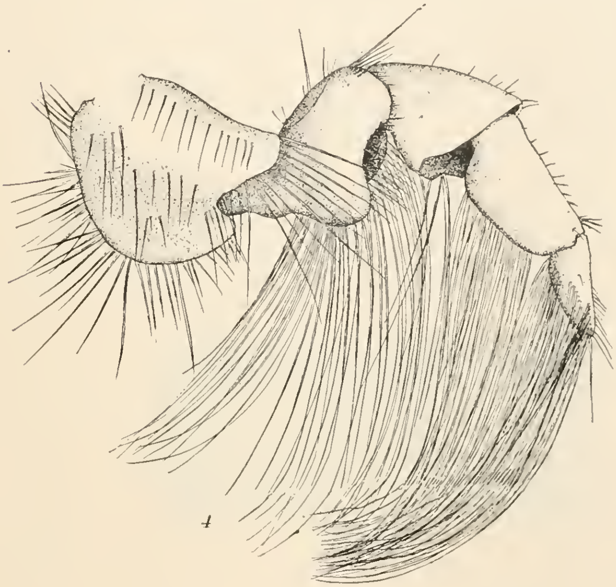
altered at once. These appendages are waved quickly at regular intervals while extended.

Polyonyx is very cleanly. The last leg is not used for locomotion, but is peculiarly modified, as in many anomurnas, to form a cleaning organ (Figs. 3, 7). The tip is chelate and is provided with peculiar pectinate and plumose setæ. The crustaceans use these appendages with great expertness; they can reach all parts of the body with them, even the interior of the gill chambers, and can frequently be seen industriously currying themselves. As a result, the body is always as smooth and clean as new porcelain. *Pinnixa* apparently takes little care for its cleanliness. Except for the mouth parts, antennæ and eye stalks, the body is usually dirty and over-grown with various organisms. A bryozoan completely covered the exposed parts of several of the individuals captured (Fig. 2*b*), a *Vorticella*-like protozoan was attached to others, and one crab was observed that carried a small clam, *Mytilus edulis* (Linnæus), attached to its last leg by byssal threads. Concerning the bryozoan Osborn ('12) says: "*Hippuraria elongata* is also a commensal living in the branchial chambers of the blue and spider crabs and on the carapace of *Pinnixa* living in the tubes of *Chætopterus*." This species is apparently not found except in association with crabs.

In most of the particulars described the two crustacean tenants in *Chætopterus* tubes showed striking dissimilarity, but their feeding habits were very much alike. Both *Polyonyx* and *Pinnixa* obtain their food by "net-fishing" (Calman, '11, p. 115) like barnacles. The "nets" in both are formed by the endopods of the third maxilliped which are well supplied with plumose setæ. A *Polyonyx* by spreading his nets (Fig. 4) can strain most of the water passing through the tube where he lives. They are extended laterally and swept together below the body. Captured food is carried against the ventral side of the body where it is scraped from the setæ of the net by the mouth-parts. *Pinnixa* feeds in a similar manner but the nets are raised above the head and swept forward and downward against the mouth. Various small organisms are captured. Eight fresh *Pinnixa* stomachs were examined on August 5 and found to contain (in order of abundance): pieces of algal filaments, diatoms, a flagellate

(*Exuvella*), fine silt, and other unidentifiable particles. Five *Polyonyx* stomachs examined August 9 contained: diatoms, silt, algal filaments, and spores or cysts. The commensals apparently feed on such organic matter as can be strained from the water passing through the worm tubes in which they live.

Enders ('05) points out that a prolonged breeding period characterizes *Polyonyx* and *Pinnixa*, and he believes this condition is due to the protection afforded by the worm tubes. Every female he took at Beaufort, from June 21 to October 25, bore eggs or had recently shed them. Every female taken by the writer at Woods Hole (July 18 to August 9) bore eggs or young. Four females shed their zoëa in the dishes where they were kept in the laboratory. The bluish eggs of *Pinnixa* are well protected by the broad abdomen which folds tight against the body and completely covers them. Those of *Polyonyx* are bright red; they project so that they are not concealed by the abdomen and appear as a bright mass between the body and the chelipeds when the crustacean is viewed from above (Fig. 2*a*).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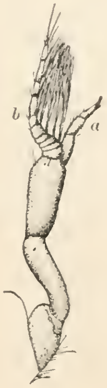

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FIG. 4. The endopod of the third maxilliped of *Polyonyx*—"the fishing net." The long setæ on the four distal segments are plumose (not shown in the figure).

FIG. 5. First antenna (antennule) of *Polyonyx*.

The light reactions of the commensals were tested. Animals were placed in a flat dish containing sea water. This dish was barely enclosed by a box two feet long and sixteen inches wide. The box was painted black on the inside and light was admitted through an aperture two inches high across the lower side of one end. When a *Pinnixa* or a *Polyonyx* was placed in the box before a window it usually went toward the light and tried to get through the glass for a time. It soon began to wander about the dish, however, and after twenty-four hours spent most of the time in the darkest end of the box. When ten individuals of the same species were put in the dish simultaneously their behavior was essentially the same. Twenty *Pinnixæ* and seven *Polyonyx* were left together in the box several days. When the lamp was lighted before the opening of the box at night most of the crustaceans went toward it and tried to get through the end of the glass dish, but after an hour they became scattered about the dish without particular reference to the light. Mast ('11, p. 284) mentions several animals which, though usually negatively phototropic, may become positive for a time after mechanical or other stimulation. Apparently steady light is not, as a rule, an important factor in the daily life of *Pinnixa* and *Polyonyx*, but if confined under unnatural conditions they may go toward a light. Such reactions might enable them to escape from confinement. Both *Pinnixa* and *Polyonyx* responded readily to a decrease in light intensity. The former was very active when placed in a glass dish and kept its legs continually in motion; but if an object was passed between it and the window it became motionless at once. Though *Polyonyx* was more

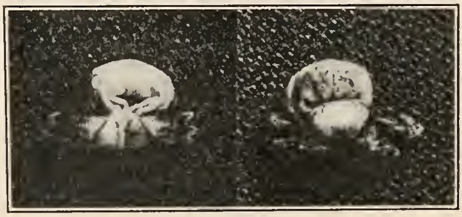

FIG. 6. *Pinnixa* undergoing ecdysis. The left-hand figure shows a ventral, the right-hand a dorsal view.

sluggish it showed similar sensitiveness to shadows. Such reactions would of course help to protect these animals from predaceous enemies.

On July 24 one of the *Pinnixæ* in the "dark box" liberated a number of zoëa larvæ which swam persistently against the glass toward the light for an entire day; they were then removed.

Pinnixæ underwent ecdysis in the dishes in which they were kept; one shed on July 24 and two on August 5 (Fig. 6). They were apparently much hardier than *Polyonyx* and did not throw off their legs readily nor die if the water became foul. *Polyonyx*, like all its near relatives (Calman, '11, p. 114), readily practices autotomy, and quickly succumbs to unfavorable conditions. Its extreme cleanliness has already been mentioned.

General Discussion.

Perhaps the chief point of interest in these dissimilar crustaceans which have come to be associated with *Chætopterus* so closely that they are rarely found elsewhere lies in the similarities in physiology and structure which have enabled them to take up such a peculiar mode of life. The similarities ought to point to essential or fundamental characteristics from an ecological point of view, and the unlike features should be of less importance.

Let us first examine the differences between *Polyonyx* and *Pinnixa*. As to relationship, both are decapods, but the former belongs to the family Porcellanidæ (tribe Galatheidea, section Anomura) and is therefore not a crab despite its general appearance; the latter is a true crab of the family Pinnotheridæ (tribe Brachygnatha, section Brachyura). The Porcellanidæ use only three pairs of legs for walking, the first forming the chelæ and the last being very small and carried folded up at the sides of the body or even within the gill chambers. They are mostly found under stones along the sea shore and among corals. The family Pinnotheridæ contains many crabs which live as commensals in molluscs, worms, sea-urchins, and other animals. Their shells are often softened or membranaceous, and their eyes are very small; both these characteristics have been supposed to have arisen as a result of commensal life (Stebbing, '93, p. 100); the fifth legs are much shorter than the fourth but are used for walking. *Pinnixa* never walks backwards, while *Polyonyx* never walks forwards; but both move sideways.

Pinnixa is very hardy and can stand foul water, as well as the indiscriminate growth of organisms on its carapace, and does not have a strong respiratory current. *Polyonyx* is not hardy, but takes every precaution to protect itself. It has a special cleaning appendage; when at rest it stands high on its setal fringe above the dirt that may collect under it; it never burrows; it has a very strong respiratory current which is deflected laterally so as to clear its abode. Apparently *Pinnixa* can endure great hardship through its great resistance, and *Polyonyx* has a number of adaptations to protect itself from contamination. The former could live almost anywhere, the latter is adapted to life in *Chætopterus* tubes or other protected situations.

The similarities between these commensals are as follows: Both are, like most crustaceans, strongly thigmotropic, and creep into crevices or tubes; they become quiet when a shadow passes over them; they feed by "net casting" after the manner of barnacles; both have a very long breeding season, producing one brood after another; and both have the last leg shortened. Their thigmotropism would easily account for their entering *Chætopterus* tubes and their feeding habits are admirably suited for the capture of food in such a situation. The quick cessation of motion when stimulated by a decrease in light might protect them from enemies when out of their tube. Enders ('05) believes that the long breeding period is an adaptation that has arisen as a result of the protected life in the worm tubes.

Shelford ('11, p. 603) says: "An animal should be associated: first, with breeding conditions; second, with the feeding conditions; third, with the conditions affecting shelter." In the present case, in fact in the case of most crustaceans, external conditions are of consequence in breeding only in limiting parents to a general region where the larva may carry on its later development, for the eggs are carried by the parent for a longer or shorter time. Some crustaceans (*Birgus*) have probably adapted their reproduction to suit a particular environment. The feeding habits of *Polyonyces* are such that they might exist anywhere in shallow water. It is apparently for protection that they have taken up life in *Chætopterus* tubes. While this in no way disproves Shelford's statement as a general proposition, the three factors would, in the present case, come in the following order; the most important first: (1) protection, (2) food, (3) breeding. Of course, if suitable conditions for breeding were absent the crustaceans would become extinct; but given the racial habit of carrying eggs and the abundance of pelagic microörganisms in littoral waters for food, reproduction takes care of itself, is fostered, in fact, and its products are unusually abundant.

Calman ('11, p. 217), in speaking of the association of Pinnotheres with molluscs, says, "The case is, indeed, an example of the difficulty of defining these two terms (commensal, parasite). At all events the Pinnotherid crabs show one of the characteristics of parasites in being to some extent degenerate in their structure. The carapace and the rest of the exoskeleton, no longer needed for protection, have become soft and membranous, and the eyes and antennules, the chief organs of sense, are very minute. As in many parasites, also, the eggs are very numerous, and the abdomen is very broad and deeply hollowed out for their reception." To this list I may also add that *Pinnixa's* fourth and fifth legs are used as hook-like claws for holding on, like the organs of fixation in many parasites.

There is no question but that the two commensal crustaceans discussed gain from their association with *Chætopterus*, but it is doubtful if the worm is benefited.

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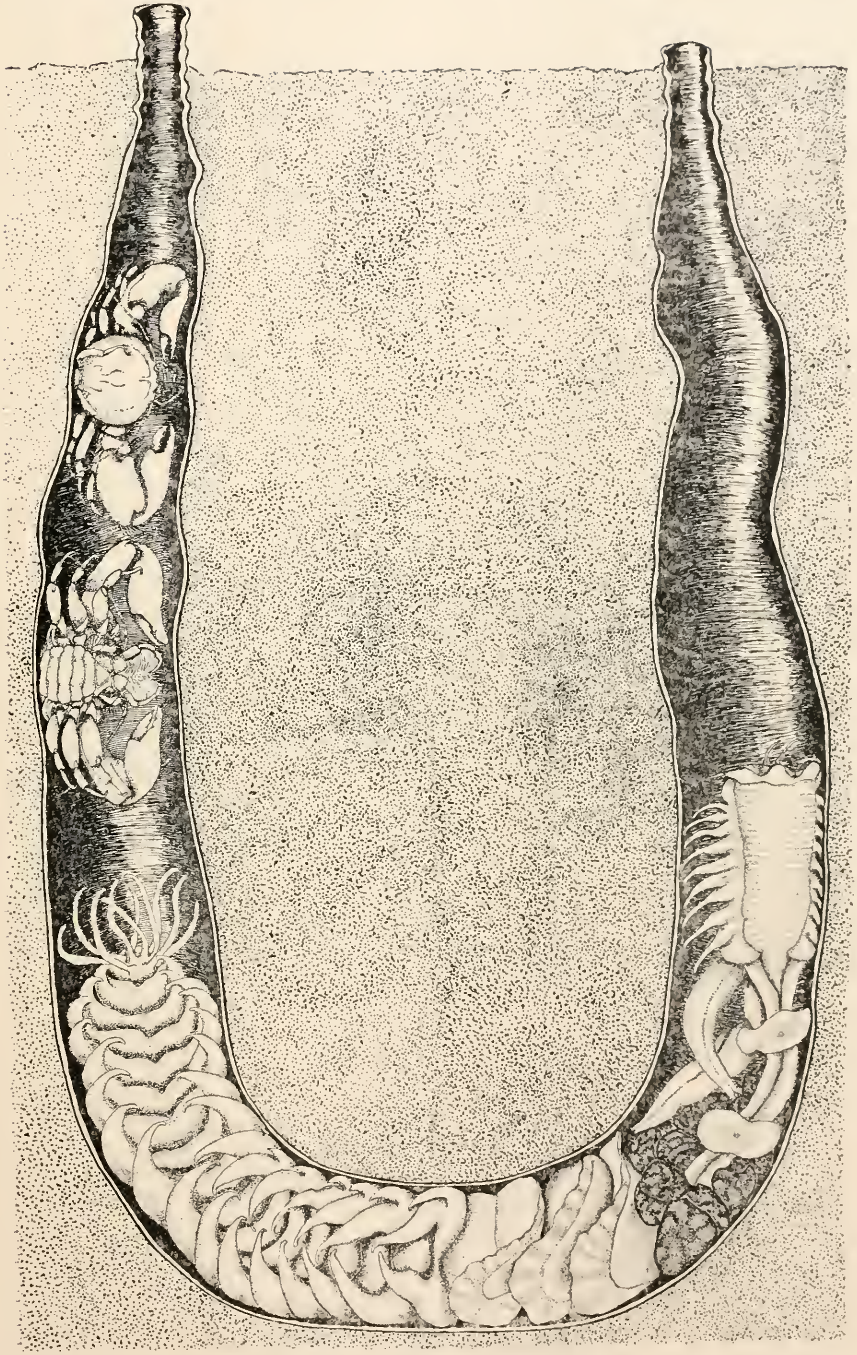
EXPLANATION OF PLATE I.

FIG. 7. Section of a *Chætopterus* tube in the sand. It contains a worm in the lower part. On the left a male (above) and a female *Polyonyx* are clinging to the wall of the tube. The male is cleaning his back with the comb on the tip of his last leg; the female rests with her ventral side toward the observer. Drawn from life by Miss Barbara Bradley.



SEX RATIOS IN DROSOPHILA AMPELOPHILA.

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As a rule there is an excess of females amongst the fruit flies that first hatch. It seemed that this must be due to the more rapid development of the females since the total output gives approximately an equal number of males and females. It was possible, although improbable, that more females are actually *produced* at first and more males later. In order to test this possibility five pairs of wild flies from stock that had been a year in confinement were placed in bottles and after two days removed to new bottles. Then after two days each pair was removed to a third bottle, etc., as long as the pair lived. Counts were made of all the flies produced in each bottle. The results showed that on an average about equal numbers of males and females came from each batch. This result occurred in the case of three of the five pairs of flies studied, as the following figures will show:

Table I.

No. of Pair.	No. of Females.	No. of Males.	Ratio.
No. 1	375	331	1 : 1
No. 2	309	281	1 : 1
No. 3	324	335	1 : 1

The fourth pair began very early to show a somewhat different sex ratio. Each day twice as many females as males hatched out, so that of the 340 individuals obtained from this pair, 222 were females and 118 were males, making a sex ratio of 2 : 1.

The fifth pair proved to be even more unusual than the fourth in its excess of females for each day two, three and even four times as many females as males would hatch out. The total number of the offspring of this pair was 439, of which 308 were females and 131 males, making a sex ratio of 2.3 : 1.

When this unusual ratio was first noticed, at the suggestion of Professor Morgan, some of the flies that hatched out were mated, the sisters with their brothers, in order to see whether the peculiar ratio of the parents would be transmitted to any of the children. Sixty-three pairs were made up and kept in good condition, each female being allowed to lay all of her eggs, and the offspring of each carefully separated according to sex and counted. Out of the 63 pairs, 26 showed various high and in some cases unexpected sex ratios, while 37 gave a normal ratio. The results are shown in the following table:

TABLE II.

Pair.	No. of Females.	No. of Males.	Ratio.
No. 1	312	3	104 : 1
No. 2	169	5	34 : 1
No. 3	276	26	10 : 1
No. 4	291	54	5 : 1
No. 5	147	32	4 : 1
No. 6	87	27	3 : 1
No. 7	86	30	3 : 1
No. 8	225	128	2 : 1
No. 9	135	61	2 : 1
No. 10	163	62	2 : 1
No. 11	289	148	2 : 1
No. 12	121	65	2 : 1
No. 13	367	166	2 : 1
No. 14	236	108	2 : 1
No. 15	151	71	2 : 1
No. 16	254	120	2 : 1
No. 17	174	85	2 : 1
No. 18	195	88	2 : 1
No. 19	100	51	2 : 1
No. 20	121	61	2 : 1
No. 21	119	54	2 : 1
No. 22	169	65	2 : 1
No. 23	195	84	2 : 1
No. 24	176	79	2 : 1
No. 25	285	135	2 : 1
No. 26	236	108	2 : 1

One of the first questions that arose was, whether the male or the female was causing the peculiar ratio and to examine this question the following experiments were carried out. The male parent of the best line showing a ratio of 104 : 1 died before I had a chance to breed him with other females, so the father of the line showing the next best ratio 34 : 1 was used instead. He was mated with five wild females, great care being taken to use only virgin females, with the following results:

Table III.

Pair.	No. of Females.	No. of Males.	Ratio.
No. 1	129	85	1 : 1
No. 2	165	173	1 : 1
No. 3	111	103	1 : 1
No. 4	120	110	1 : 1
No. 5	90	91	1 : 1

The father of another good line with a sex ratio of 10 : 1 was also crossed with two virgin wild females and the following results obtained:

Table IV.

Pair.	No. of Females.	No. of Males.	Ratio.
No. 1	89	103	1 : 1
No. 2	47	50	1 : 1

Eight males were taken promiscuously from the four lines showing the four best sex ratios and crossed with virgin wild females, with results as follows:

Table VI.

Pair.	No. of Females.	No. of Males.	Ratio.
No. 1	220	192	1 : 1
No. 2	147	127	1 : 1
No. 3	167	171	1 : 1
No. 4	103	80	1 : 1
No. 5	134	147	1 : 1
No. 6	178	148	1 : 1
No. 7	112	105	1 : 1
No. 8	115	119	1 : 1

All of these results seem to show conclusively that the abnormal sex ratio is not caused or influenced by the male and it seems probable that it is to the female we must look for a further explanation. This conclusion, therefore, eliminates one of the possible explanations for an excess of females, namely, that the male-producing sperm are non-functional.

Other experiments were carried on at the same time with the female children of the four best lines, to see if the female was causing the unusual ratios. Twelve of the virgin females were crossed with wild males, with these results:

Table VIII.

Pair.	No. of Females.	No. of Males.	Ratio.
No. 1	112	43	2+ : 1
No. 2	201	129	1.6 : 1
No. 3	110	108	1 : 1
No. 4	84	38	2 : 1
No. 5	211	103	2 : 1
No. 6	248	121	2 : 1
No. 7	189	191	1 : 1
No. 8	211	138	1.5 : 1
No. 9	57	24	2 : 1
No. 10	152	110	1 : 1
No. 11	147	61	2 : 1
No. 12	156	86	2 : 1

Thus it is shown that it is the female that produces the abnormal sex ratios, just how, is not apparent at present.

One hundred and six of the females from the best line with the 104 : 1 ratio were crossed with wild males in order to see whether any of these children had inherited an abnormal sex ratio from their mother and whether the ratio in any case would be as high as that of the mother. The results are as follows in Table IX.

Thus, although none of the children[1] inherited as high a ratio as their mother showed, at the same time almost all of them inherited a ratio above normal, the majority showing that of their grandmother.

Another generation was obtained by mating 119 of the virgin females taken from the lines showing a 3 : 1 ratio to wild males, in order to see whether the abnormal ratio still persisted and if the 104 : 1 ratio could be recovered again. The general results are as follows:

11 females showed a sex ratio of 3 : 1
48 females showed a sex ratio of 2 : 1
60 females showed a sex ratio of 1 : 1

The exact figures are given in Table X.

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[1] Eight females died.

Table IX.

Pair.	No. of Females.	No. of Males.	Ratio.
No. 1	144	56	(−3) : 1
No. 2	282	99	3 : 1
No. 3	323	112	3 : 1
No. 4	107	35	3 : 1
No. 5	201	67	3 : 1
No. 6	150	49	3 : 1
No. 7	219	75	3 : 1
No. 8	220	76	3 : 1
No. 9	106	39	3 : 1
No. 10	289	102	(−3) : 1
No. 11	277	139	2 : 1
No. 12	177	96	(−2) : 1
No. 13	316	160	2 : 1
No. 14	248	102	2 : 1
No. 15	250	101	2 : 1
No. 16	207	113	2 : 1
No. 17	173	84	2 : 1
No. 18	276	133	2 : 1
No. 19	113	64	(−2) : 1
No. 20	167	79	2 : 1
No. 21	173	79	2 : 1
No. 22	213	111	2 : 1
No. 23	120	69	2 : 1
No. 24	193	93	2 : 1
No. 25	164	72	2 : 1
No. 26	195	96	2 : 1
No. 27	204	102	2 : 1
No. 28	251	98	2 : 1
No. 29	189	72	2 : 1
No. 30	129	67	2 : 1
No. 31	209	85	2 : 1
No. 32	144	59	2 : 1
No. 33	139	57	2 : 1
No. 34	201	76	2 : 1
No. 35	109	44	2 : 1
No. 36	191	78	2 : 1
No. 37	239	101	2 : 1
No. 38	148	65	2 : 1
No. 39	129	73	(−2) : 1
No. 40	228	93	2 : 1
No. 41	317	148	2 : 1
No. 42	235	115	2 : 1
No. 43	252	121	2 : 1
No. 44	138	56	2 : 1
No. 45	154	66	2 : 1
No. 46	87	43	2 : 1
No. 47	118	50	2 : 1
No. 48	214	83	2 : 1
No. 49	181	98	2 : 1
No. 50	205	120	(−2) : 1
No. 51	254	104	2 : 1
No. 52	156	85	(−2) : 1
No. 53	211	85	2 : 1
No. 54	175	89	2 : 1
No. 55	178	70	2 : 1
No. 56	145	72	2 : 1
No. 57	123	48	2 : 1
No. 58	191	94	2 : 1
No. 59	187	66	2 : 1

TABLE IX—*Continued.*

Pair.	No. of Females.	No. of Males.	Ratio.
No. 60	167	64	2 : 1
No. 61	117	57	2 : 1
No. 62	130	63	2 : 1
No. 63	322	140	2 : 1
No. 64	129	62	2 : 1
No. 65	229	111	2 : 1
No. 66	300	123	2 : 1
No. 67	269	143	(−2) : 1
No. 68	286	133	2 : 1
No. 69	284	126	2 : 1
No. 70	240	124	2 : 1
No. 71	92	40	2 : 1
No. 72	273	118	2 : 1
No. 73	197	98	2 : 1
No. 74	70	35	2 : 1
No. 75	243	103	2 : 1
No. 76	168	63	2 : 1
No. 77	116	56	2 : 1
No. 78	210	102	2 : 1
No. 79	243	110	2 : 1
No. 80	237	108	2 : 1
No. 81	149	65	2 : 1
No. 82	124	56	2 : 1
No. 83	82	41	2 : 1
No. 84	64	28	2 : 1
No. 85	133	64	2 : 1
No. 86	122	57	2 : 1
No. 87	96	45	2 : 1
No. 88	97	54	2 : 1
No. 89	56	25	2 : 1
No. 90	35	17	2 : 1
No. 91	49	26	2 : 1
No 92	165	104	(1+) : 1
No. 93	193	118	(1+) : 1
No. 94	210	216	1 : 1
No. 95	133	85	(1+) : 1
No. 96	150	140	1 : 1
No. 97	178	107	(1+) : 1
No. 98	71	44	1 : 1

TABLE X.

Pair.	No. of Females.	No. of Males.	Ratio.
No. 1	143	49	3 : 1
No. 2	164	59	3 : 1
No. 3	124	43	3 : 1
No. 4	149	50	3 : 1
No. 5	118	39	3 : 1
No. 6	139	49	3 : 1
No. 7	143	45	3 : 1
No. 8	144	52	3 : 1
No. 9	111	39	3 : 1
No. 10	87	30	3 : 1
No. 11	91	33	3 : 1
No. 12	140	63	2 : 1
No. 13	36	17	2 : 1
No. 14	140	75	2 : 1
No. 15	141	52	2(+) : 1
No. 16	83	40	2 : 1
No. 17	66	36	2 : 1
No. 18	35	19	2 : 1
No. 19	150	68	2 : 1
No. 20	95	50	2 : 1
No. 21	176	70	2 : 1
No. 22	182	90	2 : 1
No. 23	50	23	2 : 1
No. 24	34	16	2 : 1
No. 25	83	44	2 : 1
No. 26	119	51	2 : 1
No. 27	110	40	2(+) : 1
No. 28	96	50	2 : 1
No. 29	133	74	2 : 1
No. 30	146	76	2 : 1
No. 31	51	23	2 : 1
No. 32	138	53	2 : 1
No. 33	147	71	2 : 1
No. 34	126	62	2 : 1
No. 35	153	79	2 : 1
No. 36	104	46	2 : 1
No. 37	103	44	2 : 1
No. 38	79	42	2 : 1
No. 39	147	62	2 : 1
No. 40	79	40	2 : 1
No. 41	162	77	2 : 1
No. 42	92	45	2 : 1
No. 43	98	51	2 : 1
No. 44	122	60	2 : 1
No. 45	96	52	2 : 1
No. 46	118	46	2 : 1
No. 47	133	55	2 : 1
No. 48	68	33	2 : 1
No. 49	72	37	2 : 1
No. 50	76	34	2 : 1
No. 51	105	55	2 : 1
No. 52	173	89	2 : 1
No. 53	78	40	2 : 1
No. 54	124	46	2(+) : 1
No. 55	106	50	2 : 1
No. 56	89	39	2 : 1
No. 57	139	61	2 : 1
No. 58	102	50	2 : 1
No. 59	90	50	2 : 1
No. 60	117	104	1 : 1

Table X—*Continued.*

Pair.	No. of Females.	No. of Males.	Ratio.
No. 61	75	55	1 : 1
No. 62	46	56	1 : 1
No. 63	25	25	1 : 1
No. 64	53	55	1 : 1
No. 65	27	36	1 : 1
No. 66	91	78	1 : 1
No. 67	60	59	1 : 1
No. 68	118	86	1 : 1
No. 69	110	66	1(–) : 1
No. 70	61	51	1 : 1
No. 71	44	53	1 : 1
No. 72	114	100	1 : 1
No. 73	103	64	1(–) : 1
No. 74	122	85	1 : 1
No. 75	82	57	1 : 1
No. 76	122	122	1 : 1
No. 77	109	88	1 : 1
No. 78	142	131	1 : 1
No. 79	128	132	1 : 1
No. 80	133	129	1 : 1
No. 81	97	99	1 : 1
No. 82	114	96	1 : 1
No. 83	97	77	1 : 1
No. 84	111	98	1 : 1
No. 85	102	107	1 : 1
No. 86	55	46	1 : 1
No. 87	93	87	1 : 1
No. 88	92	63	1 : 1
No. 89	98	90	1 : 1
No. 90	118	98	1 : 1
No. 91	136	141	1 : 1
No. 92	109	96	1 : 1
No. 93	118	109	1 : 1
No. 94	105	85	1 : 1
No. 95	136	104	1 : 1
No. 96	121	101	1 : 1
No. 97	133	159	1 : 1
No. 98	164	94	1 : 1
No. 99	72	77	1 : 1
No. 100	84	81	1 : 1
No. 101	98	60	1(–) : 1
No. 102	93	55	1(–) : 1
No. 103	106	106	1 : 1
No. 104	94	73	1 : 1
No. 105	80	68	1 : 1
No. 106	66	54	1 : 1
No. 107	81	85	1 : 1
No. 108	71	40	1(–) : 1
No. 109	82	80	1 : 1
No. 110	108	89	1 : 1
No. 111	91	49	1(–) : 1
No. 112	102	100	1 : 1
No. 113	93	106	1 : 1
No. 114	45	67	1 : 1
No. 115	101	79	1 : 1
No. 116	93	92	1 : 1
No. 117	88	104	1 : 1
No. 118	84	85	1 : 1
No. 119	67	70	1 : 1

Two other generations have been carried through with these results:

From 49 females mated

1 showed a ratio of 4 : 1 3 showed a ratio of 3 : 1
12 showed a ratio of 2 : 1 33 showed a ratio of 1 : 1

The exact figures are:

TABLE XI.

Pair.	No. of Females.	No. of Males.	Ratio.
No. 1	92	22	4 : 1
No. 2	97	33	3 : 1
No. 3	62	16	3 : 1
No. 4	139	50	3 : 1
No. 5	105	48	2 : 1
No. 6	95	41	2 : 1
No. 7	147	66	2+ : 1
No. 8	148	59	2+ : 1
No. 9	66	33	2 : 1
No. 10	46	21	2 : 1
No. 11	174	65	2+ : 1
No. 12	76	38	2 : 1
No. 13	172	79	2+ : 1
No. 14	74	39	2 : 1
No. 15	110	54	2 : 1
No. 16	79	41	2 : 1
No. 17	117	78	1+ : 1
No. 18	138	130	1 : 1
No. 19	96	91	1 : 1
No. 20	110	98	1 : 1
No. 21	64	60	1 : 1
No. 22	152	87	1+ : 1
No. 23	48	35	1 : 1
No. 24	162	165	1 : 1
No. 25	180	138	1+ : 1
No. 26	73	79	1 : 1
No. 27	91	89	1 : 1
No. 28	67	68	1 : 1
No. 29	117	78	1+ : 1
No. 30	148	152	1 : 1
No. 31	155	110	1+ : 1
No. 32	52	39	1 : 1
No. 33	62	46	1+ : 1
No. 34	69	44	1+ : 1
No. 35	135	114	1 : 1
No. 36	126	95	1 : 1
No. 37	78	45	1+ : 1
No. 38	100	71	1 : 1
No. 39	64	70	1 : 1
No. 40	70	64	1 : 1
No. 41	46	59	1 : 1
No. 42	136	95	1+ : 1
No. 43	63	40	1+ : 1
No. 44	61	67	1 : 1
No. 45	74	73	1 : 1
No. 46	107	76	1 : 1
No. 47	37	38	1 : 1
No. 48	96	77	1 : 1
No. 49	91	107	1 : 1

From 25 females mated with wild males

1 showed a ratio of 3 : 1
10 showed a ratio of 2 : 1
14 showed a ratio of 1 : 1

The exact figures are:

Table XII.

Pair.	No. of Females.	No. of Males.	Ratio.
No. 1	91	31	3 : 1
No. 2	69	33	2 : 1
No. 3	123	51	2 : 1
No. 4	90	47	2 : 1
No. 5	116	59	2 : 1
No. 6	104	41	2+ : 1
No. 7	90	45	2 : 1
No. 8	59	24	2 : 1
No. 9	58	26	2 : 1
No. 10	60	25	2 : 1
No. 11	53	27	2 : 1
No. 12	40	46	1 : 1
No. 13	39	57	1 : 1
No. 14	86	57	1 : 1
No. 15	96	87	1 : 1
No. 16	70	61	1 : 1
No. 17	51	48	1 : 1
No. 18	69	58	1 : 1
No. 19	59	71	1 : 1
No. 20	121	78	1+ : 1
No. 21	59	69	1 : 1
No. 22	68	57	1 : 1
No. 23	52	64	1 : 1
No. 24	94	83	1 : 1
No. 25	56	43	1 : 1

The results of these last generations seem to indicate that the unusual sex ratio is gradually disappearing, to judge from the number of individuals that inherit it, but whether it can be maintained by breeding from certain strains remains to be determined.

Vol. XXIV. *February, 1913.* *No. 3.*

BIOLOGICAL BULLETIN

THE ODD CHROMOSOME IN CERASTIPSOCUS VENOSUS.[1]

ALICE M. BORING.

Many groups of insects belonging to the species *Cerastipsocus venosus* were found on one ash tree near the laboratory at Woods Hole last summer. As they belong to the Corrodentia, an order of insects in which the chromosome history has not yet been worked out, it seemed worth while to study the spermatogenesis. They are gregarious in habit and not strong flyers. In the middle of August there were still many nymphs, but they all soon developed wings and by September 1 had all flown from the ash tree. The material used was partly from nymphs and partly from adults. The males are slightly smaller than the females, and both nymphs and adults furnished the entire series of stages for the study of the spermatogenesis. The spermatogenesis falls into line with that of many insects in other orders. There is an odd chromosome that does not divide in the first spermatocyte division.

METHODS.

The testes are small trilobed organs, resembling clover leaves, situated dorsally, one each side of the anterior end of the abdomen. The most of this study was made from acetocarmine preparations, but compared with material fixed in Gilson or Flemming and stained in iron hæmatoxylin or thionin. The small testis was put whole in a drop of Schneider's acetocarmine on a slide, left there a couple of minutes, covered with a cover-glass, pressed flat, all extra acetocarmine was drawn off with filter paper until the edges of the coverglass were dry, and then the edges were covered with vaseline to prevent evaporation. The value of such preparations has already been pointed out many times by N. M. Stevens. They can be used immediately and will often last a couple of weeks. All chromatic structures are distinctly fixed and stained, and some achromatic structures also. The size of the cells is much larger than in preserved material, due partly, as shown by Dr. Stevens,[1] to actual swelling in the acetic acid, and partly to the avoiding of the shrinkage in alcohol and xylol (Figs. 3 and 4). This method insures the study of whole cells and avoids any danger of missing parts of a cell cut off in other sections. If the material is properly pressed out under the coverglass, there will be only a single layer of cells. All the drawings but one in this paper were made from such preparations.

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[1] I wish to express my thanks to Dr. Pearl for criticizing the manuscript of this and the following paper, to the Director of the Marine Biological Laboratory for the facilities offered me at Woods Hole, and to Mr. Nathan Banks and Mr. E. P. Van Duzee for identifying the species.

Observations.

In each lobe of the testis, the stages of spermatogenesis are arranged in a series from the distal end to the end joining the duct. The spermatogonial number is 17 (Fig. 1). Spermatogonial plates were not frequent but this number was counted clearly in four plates. The size of chromosomes varies somewhat but not sufficiently to study chromosome individuality. Not even the odd chromosome can be identified in the spermatogonia.

The odd chromosome differentiates itself first in the growth stages of the primary spermatocytes. In the early spermatocytes there is a long piece of the spireme lying close against the nuclear wall, that stains darker than the rest (Fig. 2). This next becomes more condensed and shorter, appearing as several beads. Some of the different forms are shown in Fig. 5. Finally it becomes a solid round intensely staining body in the midst of a nucleus with a very lightly staining spireme (Fig. 3). It sometimes retains, however, a slightly elongated shape in prophase (Fig. 6) and even on the spindle of the first spermatocyte division (Fig. 8). A plasmosome is also present during these growth stages.

In the stages of the first spermatocyte division, the odd chromosome is easily distinguishable from the others by its single condition. In prophase there are 8 bivalent chromosomes, appearing as dumbbells or tetrads while one odd chromosome remains round or slightly oblong with no signs of approaching division (Figs. 6 and 7). In metaphase as the dumbbells and tetrads become grouped at the equator of the spindle, the odd chromosome often still lags to one side of the equator (Figs. 8 and 9). In all these prophase and metaphase groups, the chromosomes are so large and distinct that there is no difficulty in being sure of the full number, 8 dumbbells or tetrads and one odd chromosome with no sign of division. The dumbbells are probably side views of the tetrads, as Fig. 8 shows nearly all tetrads. In the equatorial plates, the odd chromosome appears off to one side (Fig. 10). In anaphase, it sometimes precedes (Fig. 11), and sometimes follows (Fig. 12) the other chromosomes to one pole of the spindle. Fig. 13 shows the telophase of this division: the odd chromosome stands at one end of the spindle in one secondary spermatocyte, with no mate in the corresponding secondary spermatocyte. In Fig. 14, the chromosomes can be counted in the two reconstructing nuclei of the two cell products of one primary spermatocyte division: 8 dumbbells on one side, 8 dumbbells and one single odd chromosome on the other. Each dumbbell in the secondary spermatocyte represents half of a tetrad. Thus the plane of the second spermatocyte division was indicated already in the prophase of the first spermatocyte division, and still is in the prophase of the second, but there is no way to tell which is the reducing division. Among these telophase stages in one individual were a few abnormally large cells in which the mitotic mechanism had somewhere failed, and there was an unreduced number of chromosomes (Fig. 15). Here there are 16 dumbbells and the odd chromosome is recognizable from its round shape and its position to one side of the group. By the time the odd chromosome gets into the equatorial plate for the second spermatocyte division it is also a dumbbell in shape and cannot be distinguished from the others. Nothing but dumbbells appear on the secondary spermatocyte spindle, so the odd chromosome must divide in this division (Fig. 16). The equatorial plates of the secondary spermatocytes show the expected difference in the number of chromosomes, some 8

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[1] Stevens, N. M., 1908, "A Study of the Germ Cells in Certain Diptera," *Jour. Exp. Zoöl.*, V., p. 359.

(Fig. 17), and some 9 (Fig. 18), but the odd chromosome is indistinguishable by either size or position. The anaphases show no chromosome lagging behind or behaving in any way differently from the others.

The behavior of the odd chromosome necessitates the conclusion that *Cerastipsocus venosus* has dimorphic spermatozoa. If half of the secondary spermatocytes have one more chromosome than the others, the spermatids and spermatozoa developing from them must also have one more chromosome. The chief interest in this study lies in the fact that it shows the existence of an odd chromosome in a species of the Corrodentia, an order in which it has not before been described, and therefore adds another order of insects to the list of those with sex chromosomes.

SUMMARY.

1. The spermatogonial number of chromosomes is 17.
2. The odd chromosome is condensed in the growth stage, first as a long beadlike structure, later as one round body.
3. There are 9 chromosomes in the primary spermatocytes, 8 bivalent and one undivided odd chromosome.
4. The odd chromosome does not divide in the first spermatocyte division.
5. There are 9 chromosomes in half of the secondary spermatocytes and 8 in the other half.
6. All the chromosomes, including the odd chromosome, divide in the second spermatocyte division.
7. Therefore, this species of Corrodentia has dimorphic spermatozoa.

UNIVERSITY OF MAINE, ORONO,
November, 1912.

All figures were drawn with a camera lucida, 1/12 oil immersion and a 12 compensating ocular, no reduction. All drawings but Fig. 4 are from acetocarmine preparations.

EXPLANATION OF PLATE I.

FIG. 1. Metaphase of spermatogonium, showing 17 chromosomes.

FIGS. 2, 3. Spermatocyte growth stages, showing plasmosome (*P*) and two stages of condensation in the odd chromosome (*X*).

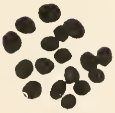
FIG. 4. Same stage as 3, but from permanent preparation fixed in Gilson.

FIG. 5. Odd chromosome in different stages of growth.

FIGS. 6, 7. Prophases of primary spermatocytes, 8 bivalents and 1 single odd chromosome (*X*).

FIGS. 8, 9. Metaphase spindles of primary spermatocytes, odd chromosome (*X*) toward one pole.

FIG. 10. Equatorial plate of primary spermatocyte, 9 chromosomes, odd chromosome (*X*) to one side of the group.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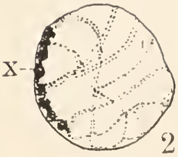

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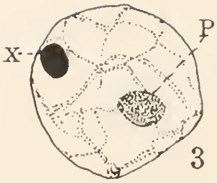

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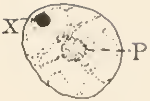

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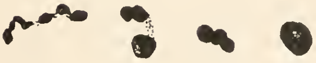
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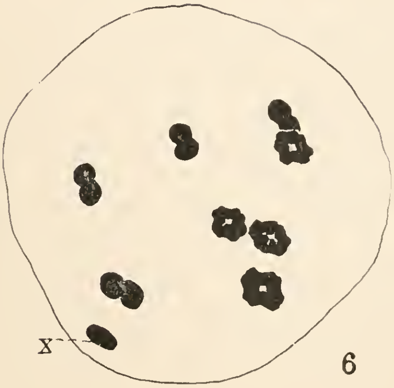

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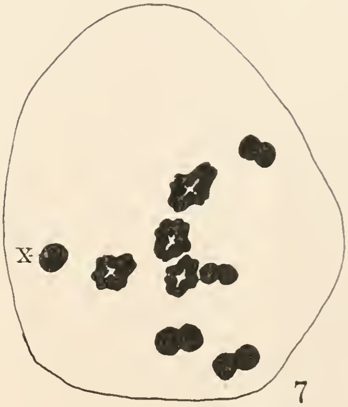

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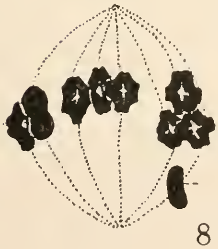
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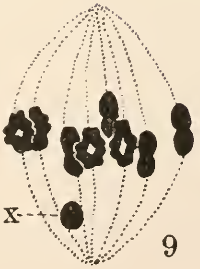

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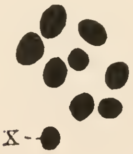

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A. M. BORING.

EXPLANATION OF PLATE II.

FIGS. 11, 12. Anaphases of primary spermatocyte division, odd chromosome (X) undivided at one pole.

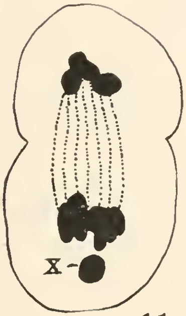
FIGS. 13, 14. Two pairs of secondary spermatocytes, X present in only one of each pair.

FIG. 15. Abnormal secondary spermatocyte, 16 chromosomes and 1 odd chromosome (X).

FIG. 16. Metaphase of secondary spermatocyte, all chromosomes dividing.

FIGS. 17, 18. Equatorial plates of secondary spermatocytes, one with 8, the other with 9 chromosomes.

FIG. 19. Anaphase of secondary spermatocyte, no lagging chromosome.



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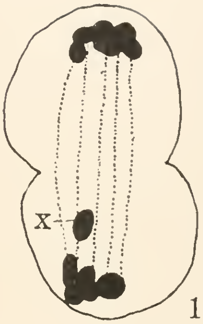

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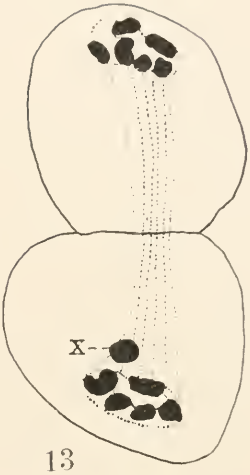

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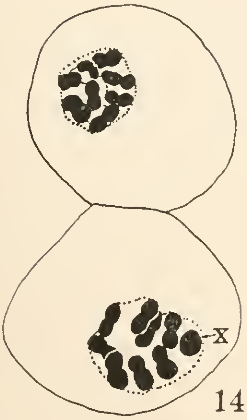

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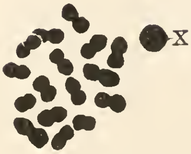

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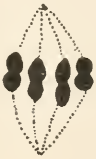

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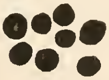

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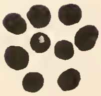

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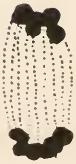

19

A. M. BORING.

THE CHROMOSOMES OF THE CERCOPIDÆ.

ALICE M. BORING.

The chromosomes of at least four species of this family have been already studied, one by Dr. N. M. Stevens,[1] and three by the writer.[2] These three species which I have already studied were collected at Cold Spring Harbor in 1907 and identified by Mr. E. P. Van Duzee as *Clastoptera obtusa*, *Aphrophora quadrangularis*, and *Aphrophora quadrinotata*. The species studied by Dr. Stevens was collected at South Harpswell in 1906 and identified at the time as *Aphrophora quadrangularis*, but the cytological differences from the Cold Spring Harbor material which was surely *Aphrophora quadrangularis* made Dr. Stevens later question the identification of the Harpswell material. The reduced number of chromosomes is different in all of these species, 12 in the Harpswell form, 15 in *Clastoptera obtusa*, 11 (12?) in *Aphrophora quadrangularis*, and 14 in *Aphrophora quadrinotata*. In other respects, the spermatogenesis in one species resembles that of the others, except for the Harpswell form which differs in several points: the odd chromosome of the early growth stages of the primary spermatocytes is long and rodlike; in later stages two M-chromosomes appear; and the odd chromosome in the equatorial plates of the primary spermatocytes is one of the medium-sized chromosomes. In the other species, the odd chromosome in the growth stages is never rodlike, but always rounded, and no M-chromosomes ever appear in the growth or division stages; the odd chromosome in the equatorial plates of the first spermatocyte division and in the sideview of the spindles appears to be one of the smaller chromosomes. In 1909, I collected material of the common European spittle insect, *Aphrophora spumaria*, at Eisenach, for chromosome study, and this summer at Woods Hole a chance to study one more species of this family was afforded me by the abundance of *Philænus spumarius* on the goldenrod and wild sunflower. The results of the study of these last two species are given in this paper.

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[1] Stevens, N. M., 1906, "Studies in Spermatogenesis," Part II., Carnegie Inst., Washington.

[2] Boring, A. M., 1907, "A Study of the Spermatogenesis in Twenty-two Species of the Membracidæ," etc., *Jour. Exp. Zoöl.*, IV., p. 469.

Material and Methods.

The *Philænus* material used in this study was found on the goldenrod and wild sunflower in the Fay woods at Woods Hole early in September. The adults were emerging rapidly from the nymph stage and the variation in marking was so striking that it seemed possible that the insects might belong to more than one species. However, specimens showing this variation were sent to Mr. E. P. Van Duzee, and he identified them all as *Philænus spumarius*. The same variations occurred among the insects on both goldenrod and wild sunflower. Nymphs from the two plants were kept separate in the laboratory until the adults appeared.

The *Aphrophora spumaria* material was collected from the grasses of a low meadow near Eisenach in September. All were adults at that time. This species exhibited as great somatic variations as *Philænus spumarius*, both in wing markings and the color of the abdomen.

The *Philænus* material used was partly from adults and partly from nymphs. The position and shape of the testes was exactly like that described for the material of the other species of Cercopidæ. In the just-emerged adults the testes show all stages of spermatogenesis, including fully formed spermatozoa, but in the females the ovaries are only slightly developed and difficult to find. There are only oögonia and very young growth stages of the primary oöcytes present. Evidently the eggs are not laid until the next spring.

For preserved material, the few posterior segments of the abdomen containing the testes were cut out and fixed in Gilson or in Flemming. But the most of the study of *Philænus* was made from acetocarmine preparations, and all the figures are drawn from such. This greatly expedited the study of the chromosomes in different individuals in connection with somatic variation. *Aphrophora spumaria* was studied entirely from preserved material.

OBSERVATIONS ON CHROMOSOME INDIVIDUALITY.

The chromosomes of *Philænus* are more like those of the three forms studied at Cold Spring Harbor than like the one worked on by Dr. Stevens at Harpswell. The abundance of the material and the large size of the chromosomes in the aceto-carmine preparations have made it possible to study the individuals in this form more accurately than previously in the other forms.

The equatorial plates of the primary spermatocytes always show 12 chromosomes (Figs. 1–7) and the secondary spermatocytes 11 and 12 (Figs. 8 and 9). The odd chromosome usually stands to one side of the primary spermatocyte plate, when it is in the same plane (Figs. 1–7, *X*), but it very often lies in an entirely different focus, at the end of the spindle (Figs. 10, 11, 12). It is always one of the smallest chromosomes. It is impossible to individualize each of the twelve chromosomes, but each plate shows one largest (*A*), two almost as large (*B*, *C*), several intermediate, and three or four smallest, one of which is the odd chromosome. The secondary spermatocyte plates show the same condition, one largest chromosome and two almost as large, but here in the plates with 11 chromosomes (Fig. 9), there is, of course, no odd chromosome and in the plates with 12 (Fig. 8), it has no particular position by which it can be identified.

The individuality of these chromosomes can be traced also in the prophase of the first spermatocyte division (Fig. 15). The odd chromosome is a single round body, while the others are already split for the first spermatocyte division. Among these dumbbell-shaped bodies the one largest and the two chromosomes almost as large are clearly recognizable (Fig. 15, *A*, *B*, *C*). In the side view of the metaphase figure the odd chromosome still appears round and undivided alongside of the other dividing chromosomes (Fig. 16). It is here about the size of half of one of the smallest bivalent chromosomes, so we could expect it to be undifferentiated by size from the smallest ones in the end view of the equatorial plates, as has been previously described.

In anaphase, the odd chromosome lags behind the others, but goes undivided to one pole. Its small size is again evident here (Fig. 17). The second spermatocyte division follows close after the first. Fig. 18 shows the two chromosome groups from the ends of a spindle. Each chromosome is splitting, preparatory to the second division, as is also the odd chromosome. The two groups were not in the same focus, and it was clear that the odd chromosome belonged to the lower group. The size relations here are as before, one largest chromosome (*A*) in each group, and two almost as large (*B*, *C*). The odd chromosome (*X*) here appears slightly larger than the smallest, but they are rearranging themselves in shape and position for the second division, and therefore we cannot be sure that we are focusing on similar sides.

The equatorial plates in the spermatogonia and in the follicle cells are interesting to compare with those in the spermatocytes, for in both of the former, the unreduced number of chromosomes is present. The grouping in the spermatogonia is more compact than in the somatic cells (Figs. 19, 20), but in both there are two longest chromosomes (*AA*) and four almost as long (*BB*, *CC*), the three pairs represented by the one largest and two almost as large of the spermatocytes. In both also there is an odd number of chromosomes, 23, the condition invariably found in the spermatogenesis of forms with an odd chromosome. Which of the smaller chromosomes here is the odd one, there is no way to detect. In one somatic cell where the chromosomes are much longer and thinner than in any others (Fig. 22), the size relations are not so clear: there are two distinctly longest (*AA*), but apparently six next longest. However, the chromosomes are so long and twisted in this case that there is every possibility of having misrepresented their lengths in a flat drawing.

The equatorial plates of the oögonia and female somatic cells are similar to the corresponding male cells, except for the possession of one more chromosome, 24 instead of 23 (Figs. 23–25). This additional chromosome must be a small one, for there are only two longest (*AA*) chromosomes and four almost as long (*BB*, *CC*), as in the male cells. Presumably then this extra chromosome is the mate of the small odd chromosome of the male. Therefore, *Philænus spumarius* has dimorphic spermatozoa, and one more chromosome in all female cells than in the male cells, one usual expression of sex differentiation in insects.

Aphrophora spumaria has one chromosome distinctly larger than the others in the equatorial plates (Figs. 26–29). Sometimes there appear to be two intermediate-sized as in *Philænus*, but the cells and chromosomes are smaller and it is consequently not possible to be sure of small differences. The reduced number of chromosomes is 12, and one of these is an odd chromosome, which behaves in the typical way in division, like *Philænus* (Fig. 33, *X*).

The Odd Chromosome in the Growth Stages.

Similar as these two species are in the number of chromosomes, and the size relations of the chromosomes, it is striking to find the odd chromosome different in the growth stages. In *Philænus*, it is sometimes slightly oval in the earliest stages (Fig. 13), but more often round, and always round in the succeeding stages (Fig. 14, *X*). This is the condition in *Clastoptera*, *Aphrophora quadrangularis*, and *Aphrophora quadrinotata*. On the other hand, *Aphrophora spumaria* has a very long narrow dark-staining odd chromosome in the early growth stages (Fig. 30), which becomes round only in the stages just before the prophase of the first spermatocyte division (Figs. 31 and 32). Just such an odd chromosome was described by Dr. Stevens for the Harpswell form. But in *Aphrophora spumaria* there are no M-chromosomes. So there is variation in the Cercopidæ as to the shape of the odd chromosome in the growth stages.

Chromosomes and Somatic Variation.

The amount of variation in markings in *Philænus spumarius* made me suspect that they might be of more than one species. Some had distinct brown lines on the wings, forming a diamond, while others were of a light tan with only a dark streak at the posterior border. As one of the important cytological problems of the present day is to connect chromosomes with somatic variations, I made a careful study of the equatorial plates of different individuals with different markings. The plates of the first spermatocytes of seven differing individuals are shown in Figs. 1–7. There is evidently no clue to somatic variation here. The plates are strikingly alike, each has one largest chromosome

(*A*), and two almost as large (*B*, *C*), and most of them show a small odd chromosome (*X*) slightly to one side in the group. In Figs. 5 and 7, it is difficult to be certain which is the odd chromosome. Two somatic cells from two of these same individuals show also the same similarity in chromosomes, two longest and four almost as long (Figs. 20 and 21, *AA*, *BB*, *CC*).

The same sort of study in *Aphrophora spumaria* gave the same negative results. The somatic variation is great, but the chromosomes give no key to it. Fig. 26 is a plate from an individual with light spotted wings, Fig. 27 from one with light tan wings with no trace of spots, Fig. 28 one with dark spotted wings and black abdomen, and Fig. 29 one with streaked wings with the abdomen black on the sternites and yellow on the pleurites. All have one largest chromosome (*A*). There are probably two next largest as in *Philænus*, but that is no distinction between insects with different somatic characters. The only chance for chromosome variation among individuals lies in the peculiar small bodies shown in Figs. 34–37. These do not always stain as deeply as the chromosomes. They resemble somewhat the supernumeraries described by Stevens[1] in *Diabrotica*, and Wilson[2] in *Metapodius*, but they do not here occur condensed in the growth stages. There may be one, two, three, or four (Figs. 34–36). Whether this number is constant for each individual, I could not tell, as the testes from several individuals with similar wings and abdomen were preserved together. My material is so limited for the study of this point that I place these few drawings here in the hope that some one where the material is available will take the trouble to collect it and work out this point more thoroughly.

SUMMARY.

1. The chromosomes of *Philænus spumarius* and *Aphrophora spumaria* were studied and compared with those of the four other species of Cercopidæ previously worked out. All have an odd chromosome which does not divide in the first spermato-

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[1] Stevens, N. M., 1908, "The Chromosomes of *Diabrotica vittata, Diabrotica soror*, and *Diabrotica 12-punctata*," *Jour. Exp. Zoöl.*, V., p. 453.

[2] Wilson, E. B., 1909, "Studies on Chromosomes," V., *Jour. Exp. Zoöl.*, VI., p. 147.

cyte division. The odd chromosome in the early growth stages of the spermatocytes varies in shape in the different species.

2. The individuality of certain chromosomes was traced in these two species of Cercopidæ. In both, the reduced number of chromosomes is 12. In *Philænus*, the identity of one large and two medium-sized chromosomes can be traced in the primary spermatocyte, secondary spermatocyte, spermatogonium, oögonium and male and female somatic cells. The odd chromosome is always one of the smallest. In *Aphrophora*, one largest chromosome always appears.

3. The somatic variation in both species is very marked, but there is no corresponding variation in the composition of the chromosome groups.

University of Maine, Orono,
November, 1912.

All figures were drawn with a camera lucida, 1/12 oil immersion and 12 compensating ocular, then reduced in the engraving to four fifths. The drawings of Plates I., II., and III. are all from acetocarmine preparations, but those of Plate IV. are from material fixed in Gilson and stained in iron hæmatoxylin.

Explanation of Letters.

A = the largest chromosome.

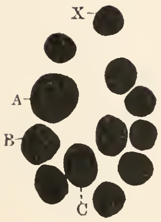
$\left.\begin{matrix} B \\ C \end{matrix}\right\}$ = the two chromosomes almost as large as A.

X = the odd chromosome.

Explanation of Plate I. (*Philænus spumarius*).

Figs. 1–7. First spermatocyte metaphases from seven individuals with different somatic markings. All show 12 chromosomes, one largest (A) and two almost as large (B, C). Most of them show the odd chromosome at one side of the group (X).

Figs. 8–9. Metaphases of the secondary spermatocytes, one with 12 and one with 11 chromosomes.



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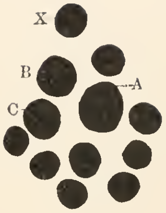

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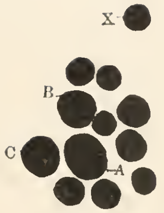

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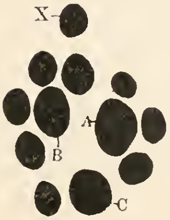

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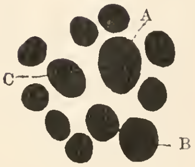

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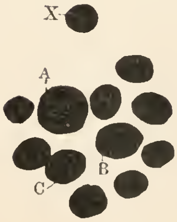

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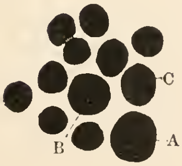

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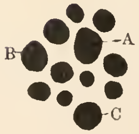

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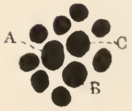


9

A. M. BORING.

EXPLANATION OF PLATE II. (*Philænus spumarius*).

FIGS. 10, 11. Two optical sections of one primary spermatocyte spindle such as is represented in Fig. 12, odd chromosome toward one pole.

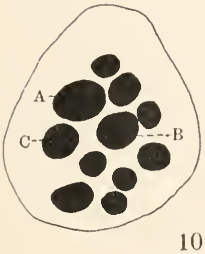
FIGS. 12, 16. Two metaphase spindles of the primary spermatocyte division.

FIGS. 13, 14. Early and late growth stages of the primary spermatocyte.

FIG. 15. Prophase of the primary spermatocyte, odd chromosome the only univalent one.

FIG. 17. Anaphase of the primary spermatocyte division, odd chromosome toward one pole.

FIG. 18. Two daughter plates of a primary spermatocyte division, each with 11 chromosomes, the odd chromosome between them.



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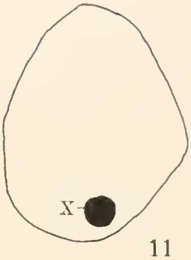

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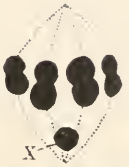

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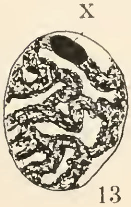

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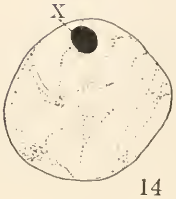

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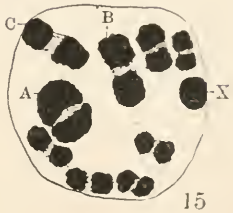

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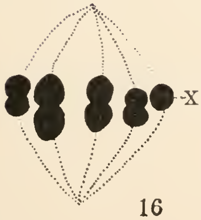

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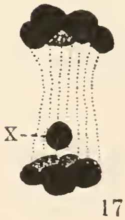

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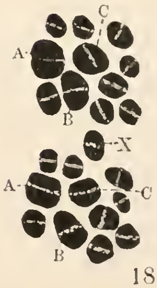


18

A. M. BORING.

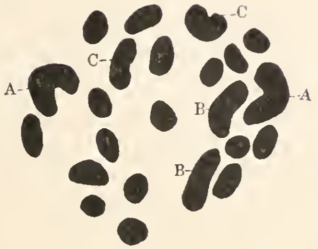
EXPLANATION OF PLATE III. (*Philænus spumarius*).

FIG. 19. Equatorial plate of a spermatogonium, 23 chromosomes, 2 largest (*AA*) and 4 next in size (*BB*, *CC*).

FIGS. 20–22. Male somatic cells, 23 chromosomes, size relations the same as in Fig. 19.

FIG. 23. Equatorial plate of an oögonium, 24 chromosomes, same size relations as in Fig. 19.

FIGS. 24, 25. Female somatic cells, 24 chromosomes, same size relations as in Fig. 19.



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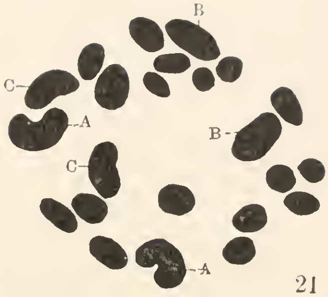

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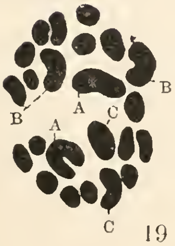

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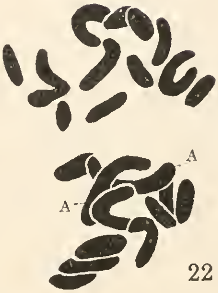

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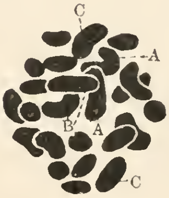

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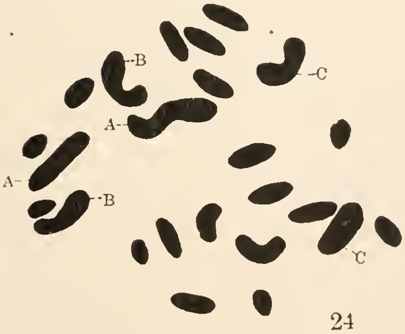

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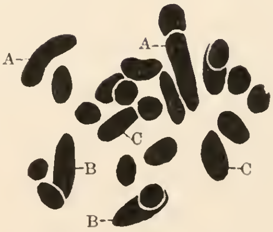


25

A. M. BORING.

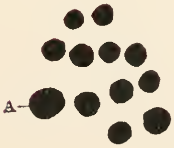
EXPLANATION OF PLATE IV. (*Aphrophora spumaria*).

FIGS. 26–29. Equatorial plates of primary spermatocytes of four different individuals with different somatic markings, one largest chromosome in each (A).

FIGS. 30–32. Growth stages of primary spermatocytes.

FIGS. 33, 35–37. Anaphases of primary spermatocytes, X toward one pole.

FIGS. 34–37. Drawings showing small chromatin bodies, which may be supernumeraries.



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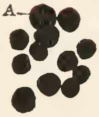

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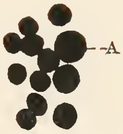

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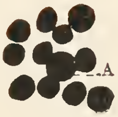

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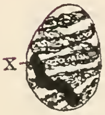

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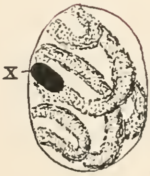

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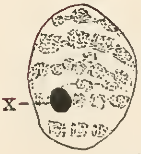

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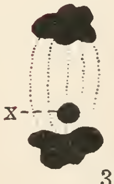

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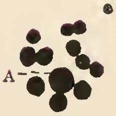

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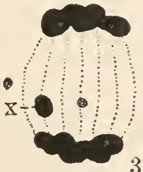

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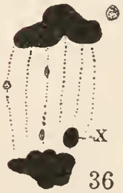

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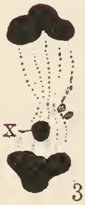

37

A. M. BORING.

BREEDING HABITS OF THE HETERONEREIS FORM OF NEREIS LIMBATA AT WOODS HOLE, MASS.

FRANK R. LILLIE AND E. E. JUST.

The senior author has used the eggs of the pelagic form of *Nereis limbata* in studies on fertilization for several years. During the seasons of 1911 and 1912 collections were made each night by the junior author, and records were kept which reveal considerable uniformity in swarming periods in accordance with the phases of the moon. In addition advantage was taken of the opportunity to study certain other features of breeding behavior. These observations are brought together in a paper partly for the sake of such general interest as they may possess and partly for the information of those who may have occasion to use this form for investigations.

The animals may be taken after sunset on certain nights, in general during the "dark of the moon," in the months of June, July, August and September. They appear swimming near the surface of the water very soon after sunset, and may be attracted by the light of a lantern and readily caught with a small hand net. The swarming usually begins with the appearance of a few males, readily distinguished by their bright red anterior segments and white sexual segments, darting rapidly through the water in curved paths in and out of the circle of light cast by the lantern. The much larger females then begin to appear, usually in smaller numbers, swimming laboriously through the water. Both sexes rapidly increase in numbers during the next fifteen minutes, and in the case of a large swarm there may be hundreds of males in sight at one time, though the number of females to be seen at one time rarely exceeds ten or a dozen.[1] In about 45 minutes the numbers begin to decrease and in an hour, or an hour and a half, all have disappeared for the night. Next night the scene may be repeated. The females are a fresh crop each night, as will be seen from the description below, though the same males may presumably appear on several successive nights, a circumstance which, if true, will partly explain the usual great numerical preponderance of males.[2]

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[1] At the height of some runs, as for instance, on the evenings of June 12, July 5 to 8, and August 6–10, 1912 (see curves 6, 7 and 1), great numbers of females appeared. On one occasion at least a liter of females was secured in ten minutes, several being caught with each sweep of the net. Within the circle of the light on such nights as many as fifty females may be seen at once.

In any given night the males invariably appear first. As the females appear in increasing numbers, the males gradually grow fewer. During the first nights of a run, as well as towards the end, the males are relatively more abundant. On nights of the greatest swarms, when the females appear in great numbers, the disproportion is perhaps not as noticeable. It seems, however, that there is no definite place in the run at which this sex ratio changes, although there are some nights throughout the summer when more females than males are caught.

During the light of the moon, with exceptions noted beyond, no *Nereis* are to be found. They therefore occur in four periods or "runs," during the summer, corresponding to the lunar cycles in the months of June, July, August and September. Each run begins near the time of the full moon, increases to a maximum during successive nights and sinks to a low point about the time of the third quarter, then again rises and falls to extinction shortly after the new moon. Each run is therefore typically divided in two sub-runs, or the curve of nightly numbers during a run is bimodal, with a deep depression about the time of the third quarter.

When a female appears she is soon surrounded by several males, if the latter are abundant, which swim rapidly in narrow circles about her. In a little while they begin to shed sperm, rendering the water milky, and soon she begins to shed her eggs, shrinking in bulk as she does so, until, a mere shadow of her former self, she sinks slowly in the water to die. We have never succeeded in keeping females alive for more than a few days after the eggs are shed.

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[2] The worms are eaten voraciously by certain fish, as we ascertained in 1911 when *Nereis* were kept in aquaria with *Fundulus*. During August, 1911, it was often noted that fish attracted by the light seemed to keep down the number of *Nereis* caught. The slowly moving females were always more easily caught by the fish. This may therefore be another cause that influences the sex ratio.

If the animals are to be kept over night for work the next day, they should be placed in separate finger bowls, preferably one for each individual, but in any case never mixing the sexes. If then the bowls are placed in a cool place, as on a water table with water flowing around them, a considerable proportion of the females and all of the males will retain their sexual products over night, and the eggs may be fertilized as desired. One great advantage of *Nereis* for experimental work is that each swarming individual is always fully mature, and contains no immature sexual elements whatever.

I. The Causes of the Sperm-shedding and Egg-shedding Reflexes.

An incidental observation of the senior author put us on the track of the immediate cause of the sperm-shedding reflex. It should be noted that males retain the sperm very tenaciously as a rule, even after days in the laboratory. One day a male was dropped accidentally in a bowl of sea water which had previously contained a female. He immediately began to shed sperm, and swam round and round in the bowl very rapidly casting sperm until the entire 200 c.c. was opalescent. This suggested that the effect of the presence of the female upon the male was exerted not through sight or touch, but through some chemical stimulus, and a few simple experiments demonstrated this conclusively.

If a ripe female be kept in a finger bowl containing about 125 c.c. of sea-water for several hours, the water will usually be found to be charged with a substance which immediately incites the sperm-shedding reflex of the male. Thus on June 17, 1911, the following test was made with about 125 c.c. of sea-water in which a female had been kept over night. A small quantity of this charged sea-water was placed in a Syracuse watch crystal, and a male was dropped in (8:15 A.M.); at once he shed sperm. He was then returned to fresh sea-water and ceased shedding. At 10:30 he was again dropped into a watch crystal of the charged sea-water and again shed sperm; in a few seconds he was transferred to fresh sea-water, and he ceased shedding sperm. Again at 2:07 he was transferred to another supply of the charged sea-water, and again shed sperm. Returned to fresh sea-water he ceased. At 2:20 he was tested again with positive results for the fourth time, and a little later was tested once more with water charged by another female and shed sperm once more.[1]

Thus each time that this animal was placed in sea-water that had contained a female for a certain length of time he responded instantaneously, and each time that he was removed and put in fresh sea-water he ceased to shed sperm.

This reaction is almost mechanical in its regularity, and can be secured with almost any male in good condition. Cases were found in which certain females apparently did not give off the effective substance, but they were very rare. As a general thing a large fully ripe female will so charge 125 c.c. of sea-water in an hour's time that any active mature male will give the sperm-shedding reflex almost instantaneously on being dropped in after the removal of the female, whereas even repeated transfers from one bowl of fresh sea-water to another causes no such response. If a male and female be placed together in a bowl of fresh sea-water they appear to stimulate one another very quickly, but it is usually several minutes, at least in the case of animals that have been kept in the laboratory over night, before the male begins to shed sperm; and the female never sheds her eggs until after the male has begun to shed sperm.

We have seen that some kind of emanation from the female incites the male to shed sperm. In the case of the female, however, it is not any comparable emanation from the male, but the presence of sperm in the sea-water that incites the shedding of eggs. The females are much more apt to shed their eggs spontaneously than the males to shed sperm. Apparently, when fully mature, many females are unable to hold the eggs back. But, in the case of those that do, it is impossible to cause the egg-shedding reflex by putting them in water that has contained males; on the other hand, the addition of a certain quantity of sperm to the water containing the female will usually cause shedding of the eggs.

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[1] Mechanical shock, killing fluids, alcohol and fresh water, may cause the worms to shed their sexual products; probably any sufficiently strong shock may act similarly. But by slow addition of alcohol to the sea-water the animals may be stupefied and die extended without shedding the sexual products. Worms freshly caught react more surely to secretions of the female than those that have been in the laboratory over 18 hours. The reactions are not so definite in the late afternoon of the day after capture, and this is associated with a general lowering of tone.

There is thus a very precise form of sexual behavior which was tested and demonstrated repeatedly during the seasons of 1911 and 1912. No attempt has been made to study the subject at all exhaustively as our attention was directed to other problems, but a few experiments were made dealing with the male reflex that throw some more light on the matter.

In the first place it is probable that the emanation of the female that incites the male to shed sperm comes mainly from the eggs. This could be shown in two ways: (1) Spent females, *i. e.*, those that have shed their eggs, do not produce the inciting substance in quantities comparable to the production of egg-bearing females. It was possible to obtain some effects by keeping four or five spent females in a small quantity of sea-water over night; but the effect on the male was so slight as probably to be attributable to the few remaining eggs in the female, or to egg secretions persisting in their bodies. Moreover mature males would not respond to the presence of immature atokous females. (2) The eggs alone produce the inciting substance quite rapidly; thus in one experiment about one third of the eggs of a single female charged about 10 c.c. of sea-water in 80 minutes so that a male shed sperm quite freely on being put in some of this water. Transferring to successive bowls of sea-water caused no reaction. The same male shed yet more freely on being transferred to the same quantity of sea-water from a bowl of 125 c.c. in which a mature female had stood over night. This was repeated with several variations always with positive results.

The tests showed that the eggs alone were considerably more effective than the spent females. The production of the stimulating substance should therefore be attributed mainly to the eggs.

The problem of the nature of the emanation that stimulates the males has received inadequate attention; but some experiments were made which narrow the field down considerably. In the first place the reaction is not due to the CO_2 excretion of the females. This follows from the fact that males do not produce any stimulating substance, though they excrete CO_2. A series of tests was also made with various dilutions of sea-water supersaturated with CO_2, all to no effect.[1] In the next place it is not due to any substance common to the females of marine animals. Tests were made with sea-water charged in one case with the emanations of *Fundulus*, in another case with the emanations of another polychæte, *Chætopterus*. Males placed in portions of such charged sea-water gave no reaction whatever, but the same males placed in a portion of sea-water that had contained a female *Nereis* over night immediately shed sperm abundantly. The mature males of *Nereis limbata* will not react even to the ripe pelagic females of a nearly related form (*N. megalops*).

It is therefore probable that the substance in question is specific. In this there is involved no new biological principle. It is well known that the males of certain butterflies, for instance, are attracted even from long distances to the females by virtue of some air-borne emanation that is certainly specific. And the same principle must also be involved in the relations of males and females in many other instances.

Another property of the exciting substance is its lability. This is shown by its disappearance on standing in the course of about three days, and by its destruction by heat. As regards the first point, the following experiment may be noted: June 24, 1911. Four samples of sea-water charged by *Nereis* females were tested, each of which had been effective when fresh: *a* was 7 days old, *b* 5 days, *c* 3 days, *d* was fresh. Of these only *d* was effective in causing male *Nereis* to shed sperm. Each of the samples *a*, *b*, and *c*, had been tested previously at intervals and each was found gradually to lose its stimulating power in the course of about 3 days. As regards the heat-lability of the substance in question, a series of experiments have shown that it requires about 10 minutes boiling to destroy the power of an effective solution entirely. Five minutes boiling diminishes the effect considerably. The ineffectiveness after boiling is not due to the removal of oxygen, for it is impossible to restore its effectiveness by aerating.

The stimulating substance is either neutralized by the presence of sperm in the water owing to combination with some sperm substance, or it ceases to be effective in the presence of a certain concentration of sperm. This is shown by the following experiment, June 20, 1911: A male put in about 8 c.c. of the water charged by a female immediately shed sperm which was stirred up by his movements; in a little while he stopped shedding. He was then transferred directly to another equal amount of the original charged water and the performance was repeated. A third transfer gave the same result, and on the fourth transfer a small amount of sperm only was shed, the male being practically spent by this time.

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[1] See footnote, p. 150.

The same phenomenon was observed on other occasions. The explanation is, I believe, that the stimulating substance is a sperm agglutinin produced by the eggs, which enters into combination with the spermatozoa and thus disappears from the solution in the presence of a sufficient quantity of sperm. The reasons for this opinion are: (1) The sea-water charged by the female does, as a matter of fact, contain a sperm agglutinin which can be shown to be neutralized by the addition of a sufficient quantity of sperm. (2) This sperm agglutinin possesses about the same degree of heat lability as the substance effective in causing the sperm-shedding reaction of the male. The relations of the sperm to egg-secretions will form the topic of another paper, but they are summarized in a preliminary paper by the senior author in *Science*, N. S., Vol. XXXVI., p. 527. The conclusion to which we have come is, therefore, that the same egg-secretion of *Nereis* which agglutinates the spermatozoa is the effective stimulus in the sperm-shedding reflex of the male. This is the simplest explanation of the facts and may be held until some reason is shown for believing that different substances are involved. Such a condition is particularly well adapted to secure fertilization under the conditions of the breeding habits of *Nereis*.

II. DATA ON THE "RUNS" AND THE "SWARMS."

By a "run" we mean an entire lunar cycle of the pelagic "*Heteronereis*"; by a "swarm" the nightly occurrence. A general statement of the facts was given in the introduction. The precise data with some comparisons follow:

1. *The Swarms.*—The swarms consist only of fully mature males and females; indeed we never secured even a single swarming animal which was not fully mature, or which contained any immature sexual elements mingled with the mature ones. The occurrence of swarming is dependent more upon the lunar cycle than any other factor; the relations with reference thereto are given under the second heading, "*The Runs.*" The second most important factor in swarming is time of day; swarming begins invariably soon after twilight and continues for an hour or two at the most. A third factor is weather; very bad weather with heavy wind and rain may prevent swarming, or at least prevent the animals from coming near enough to the surface to be seen. A fourth factor is light, partially included, of course, under the second factor. But we have reference here especially to conditions that occur at the time of full moon or a little later, when swarming may begin after twilight, and be suddenly cut short by the appearance of the moon above the eastern hills.[1] On the other hand during the light of the moon, *i. e.*, from shortly after new moon until near the time of the full moon the animals do not swarm even if the moon is entirely concealed by thick clouds.[2] The stage of the tide whether high or low was not an observable factor at our station, where the range of tidal movement is only about 18 inches. Neither do the summer variations in temperature appear to be an important factor; in any event the temperature of the water varies but little during the swarming season.

We have gained the impression that all the absolutely mature animals of any given locality swarm as soon as the right conditions are offered. This would involve the assumption that the maturing of the animals is dependent on some relation of the life history to the phases of the moon, involving probably, through lunar tidal variations, rhythmical alterations of conditions of nutrition. (Cf. Hempelmann, 1911.) This assumption at least explains fairly well the conditions as we find them.

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[1] An exception to this was noted at the time of full moon in July, 1912, when after moonrise both males and females were abundant. (See curve 1, July 28 and 29.)

[2] As an exception it may be noted that the end of the June run (1912) extended to within three days of the full moon (see curve 6). But the general rule is as stated. Special attention was directed to this point both in 1911 and 1912. There were several cloudy nights during the light of the moon in these years about the time of the first quarter of the moon; for instance, on July 21, 1912, a hard rain fell in the afternoon and the evening was dark and cloudy, but no worms could be found. We often tried collecting in dark places under wharves, etc., on moonlit nights, but always without success.

2. *The Runs.*—The collector receives very definite impression concerning the size and composition of swarms and the general features of the runs. But to give these quantitative expression is not a simple matter, though it is essential in order to reveal the order that exists. Moreover, any quantitative system of representing the data must be based on some one feature to the exclusion of others, and it is of course limited to this extent. As a result of experience we finally decided in the first place to limit the collections used in the tables to one station, and in the second place to record only females caught. The latter provision is due to the relatively enormous number of the males on certain nights which makes accurate estimation out of the question. The collecting was done each evening from the same station in the same way as nearly as possible. Practically complete records were kept for 1911 and 1912, and these have been tabulated in the form of curves in which the ordinates represent the number of females caught and the abscissæ represent days from the first quarter of the moon.

Of these records of runs we may select that of August, 1912, as being in some respects the most typical (see curve 1). It begins on the fifth day after the first quarter of the moon (July 25), and is quite atypical in this one respect, for no other run began earlier than the eighth day. On the fifth day two feamles were caught, the sixth day one, seventh day ten, eighth day eight, then the catch gradually ascends to a maximum on the twelfth day. The numbers in the successive swarms then rapidly diminish to the 15th day, when only three females were caught, but on the 16th day there were sixteen females, and on the 17th, 18th, 19th, 20th, and 21st, over thirty. The numbers then gradually decreased to the 26th day, which was the last day of the run. This run had been preceded by eight days in which no *Nereis* occurred, and it was followed by a barren period of eleven days It was therefore sharply separated from preceding and succeeding runs.

The other runs may be compared with this one as follows:

1. June, 1911; curve 2. The first collection was made on June 12, one day after the full moon or ten days after the first quarter. Judging by experience this was probably the second or third day of the run. That night 12 females were caught. The curve of this run is bimodal, sinking to zero on the 20th day from the first quarter, and then rising again. The last swarm of the run was on the 28th day after the first quarter. There is a depression in the first mode due to some unknown cause. This run was separated from the following July run by eleven days in which neither sex could be found.

2. The July, 1911, run (curve 3) began on the ninth day after the first quarter of the moon, thus on the night of the full moon (July 11). The curve of this run is also bimodal with a deep depression from the 17th to the 21st days; the last swarm was on the 26th day. Here also there is a depression in the first mode. There was an interval of eleven days from the last swarm of the July run to the first August swarm.

3. August, 1911. The run (curve 4) began on the eighth day after the first quarter and lasted to the 26th day. While the curve is decidedly irregular it is nevertheless distinctly bimodal, with a depression to zero on the 17th day. Seventeen days elapsed before the beginning of the September run.

4. September, 1911. The run (curve 5) began on the thirteenth day after the first quarter. The swarms were extremely small and few in number. But parts of the two modes of the usual curve may be recognized.

5. June, 1912; curve 6. The first worms were secured on the 13th day after the first quarter of the moon. I regard the collections from the 13th to the 17th days as constituting the first mode of the curve. The second mode is very unusual, first in its irregularity, and second in its duration; it continued in fact ten days after the new moon, and it exceeds all other records in this respect by six days. There was thus an interval of only four days between the end of June and the beginning of the July run in 1912. The last seven days of the June run thus occurred during the "light of the moon," a circumstance which must be regarded as significant. Swarming can thus take place during moonlight. Its usual absence must therefore be inter-

preted to mean that completely metamorphosed animals usually do not occur during this time.

6. July, 1912; curve 7. The run began on the ninth day after the first quarter of the moon, on the night of the full moon (June 29). This run shows no evidence of two modes, for the lack of animals on the second night of the run cannot be interpreted as the intermodal depression. The run continued until the 27th day. The absence of two modes in this case may be attributed to overlapping of the two sub-runs making up each run. This run was followed by eight days without any swarms. The August, 1912, run, already described, finished on the 26th day after the first quarter; and there was an interval of eleven days before the beginning of the September run.

7. September, 1912; curve 8. This run began on the eighth day after the first quarter of the moon, and ended unusually early on the 23d day. Though the curve is irregular in its first part there is some evidence of bimodality.

The two features that stand out most prominently in these records are the relation of the runs to the lunar cycles, and the double character of each run. The following tabulation brings out the first point; the days being numbered from the first quarter of the moon in each case:

1911. First run, tenth to twenty-eighth day.
Second run, ninth to twenty-sixth day.
Third run, eighth to twenty-sixth day.
Fourth run, thirteenth to twenty-first day.

1912. First run, thirteenth to thirty-fourth day.
Second run, ninth to twenty-seventh day.
Third run, fifth to twenty-sixth day.
Fourth run, eighth to twenty-third day.

As regards the second point, the curves show sufficiently well that each run is made up of two sub-runs. But they are not usually sharply separated by an interval as in the case of the main runs; however, the general rule is that the interval occurs near the time of the third quarter of the moon. Thus the low points in the runs as tabulated are:

1911.	Days after First Quarter.
First run	20
Second run	17–21
Third run	17
Fourth run	15–21

1912.	
First run	16–17
Second run	?
Third run	15
Fourth run	15

The third quarter of the moon comes 16–17 days after the first quarter.

Hempelmann (1911) finds that the swarms of *Nereis dumerilii* at Naples tend to center around the time of the first and third quarters of the moon; none were caught on the days of the new moon or the full moon. Thus, the monthly curve is bimodal as in the case of *Nereis limbata*, but the modes, occurring at the time of the first and third quarters, do not coincide, but alternate with those of *N. limbata* which approximate the times of the full moon and the new moon. The animals were secured from the daily plankton catch taken in the early morning hours, so little is known about the duration of the swarming or the time of its maximum. But the indications are that the time of the daily swarm is early morning (before sunrise?), and this furnishes another contrast to *N. limbata*. The runs of *N. dumerilii* occurred at Naples from October to May.

According to Akira Izuka (1903) the Japanese palolo (*Ceratocephale Osawai*) swarms closely following the new moon and the full moon. The swarms occur shortly after sunset and last an hour and a half to two hours. Each run is limited to three or four nights in the months of October and November. Thus there are two entirely distinct runs each month in this case corresponding to the sub-runs in the case of *Nereis limbata*. About ten days after each swarming period transitional half-epitokous forms are found in the bottom mud, which finish their metamorphosis in time for the next swarming period, after which only immature animals are to be found in the mud. The periodic swarming is thus dependent on periodic maturation of the animals. In many respects the swarming of *Ceratocephale* is more like that of the Woods Hole *Nereis* than any other form.

The breeding habits of the Pacific palolo (*Eunice viridis*), the Atlantic palolo (*Eunice furcata*), and *Odontosyllis* (Galloway and Welch, 1911), are similar with respect to periodicity dependent on lunar phases, though of course different in many details.

Some other annelids (*e. g.*, *Amphitrite;* see Scott, 1909) exhibit a lunar periodicity in breeding habits. The periodicity in *Nereis limbata* thus belongs to a large class of phenomena, which includes breeding seasons of some plants as well as many other animals, for which no adequate general cause has yet been found.

III. The Life History of *Nereis limbata*.

This subject is still under investigation. It is obvious that interpretation of the swarming behavior must wait for fairly complete knowledge of the life history. The present indications are that the worms either do not mature in the atokous condition, or, if they do so, that it is at an earlier time of year than our collections were made. The atokous forms were secured both in scrapings from piles of wharves and also by digging, and were kept under observation in the laboratory. All that matured underwent metamorphosis to the epitokous condition. Despite repeated efforts we failed to rear mature worms from artificially fertilized eggs. However, a few were raised to young worms about 5 mm. in length, about eight weeks after fertilization; but all of them died after an attempted change in culture methods.

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GENERAL DESCRIPTION OF CURVES.

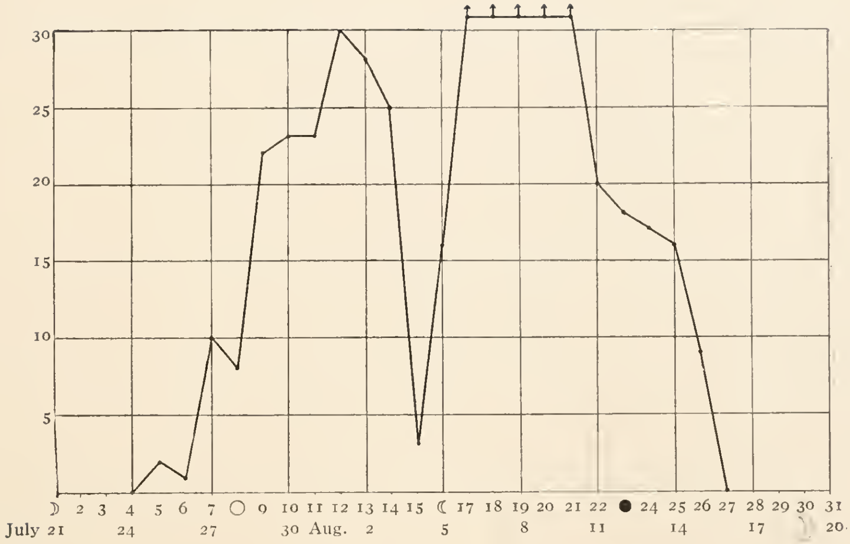
The ordinates represent the number of females caught.

The abscissæ represent days from the first quarter of the moon. The various phases of the moon are indicated. The days of the month are given in the second horizontal row of figures below the curves.

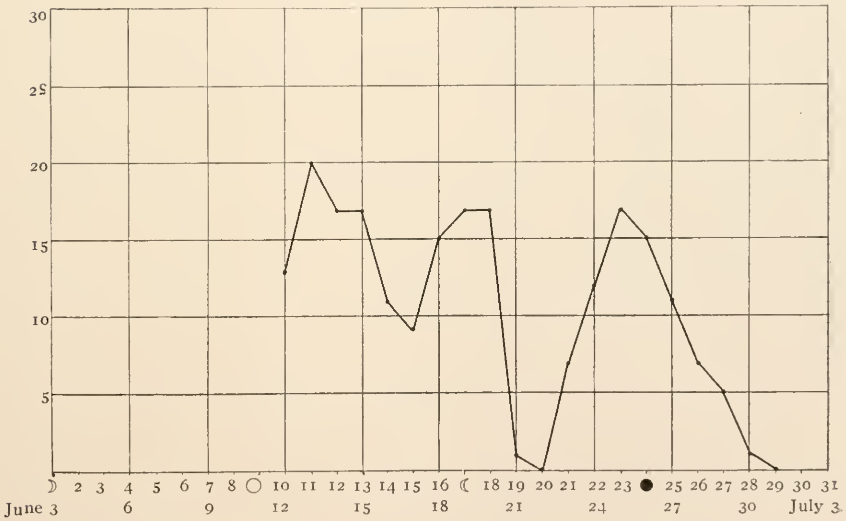
CURVE 1. The August run, 1912.

CURVE 2. The June run, 1911.

CURVE 1



CURVE 2



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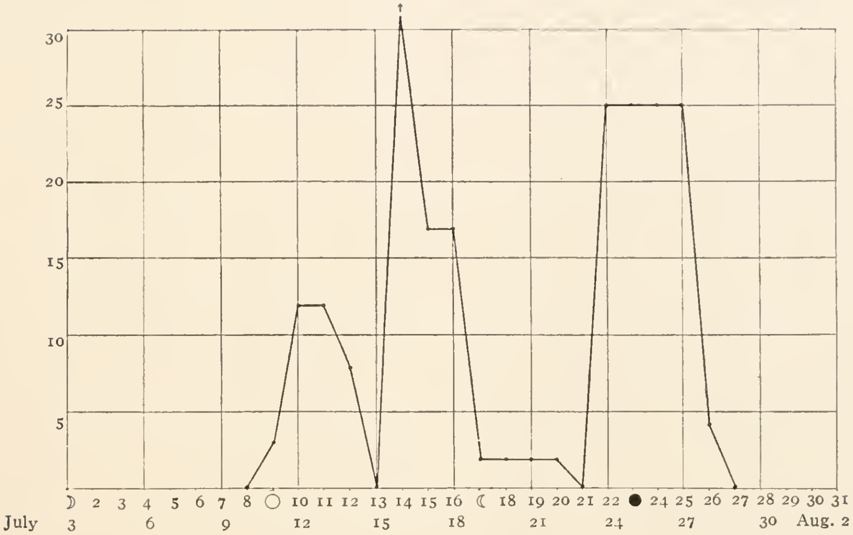
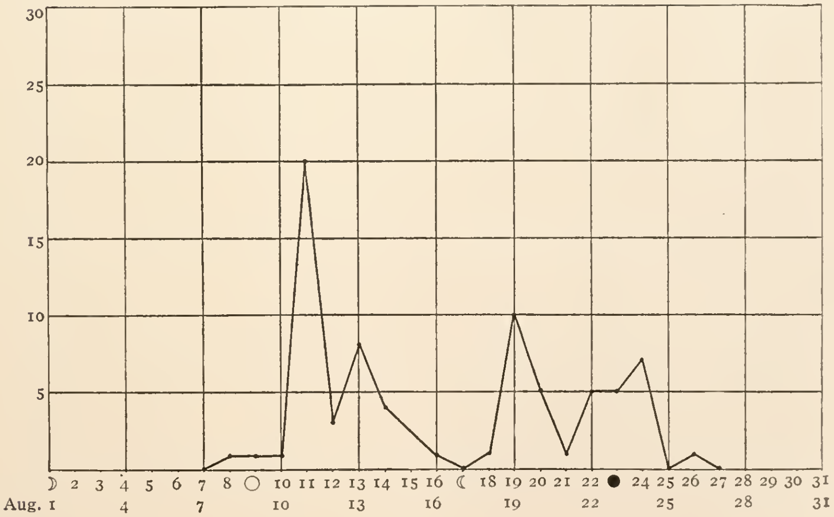
Curve 3. The July run, 1911.
Curve 4. The August run, 1911.

CURVE 3



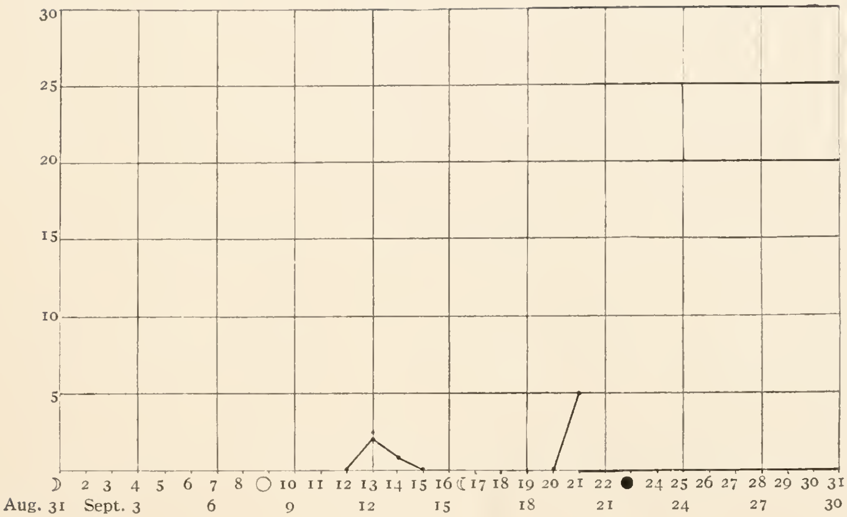
CURVE 4



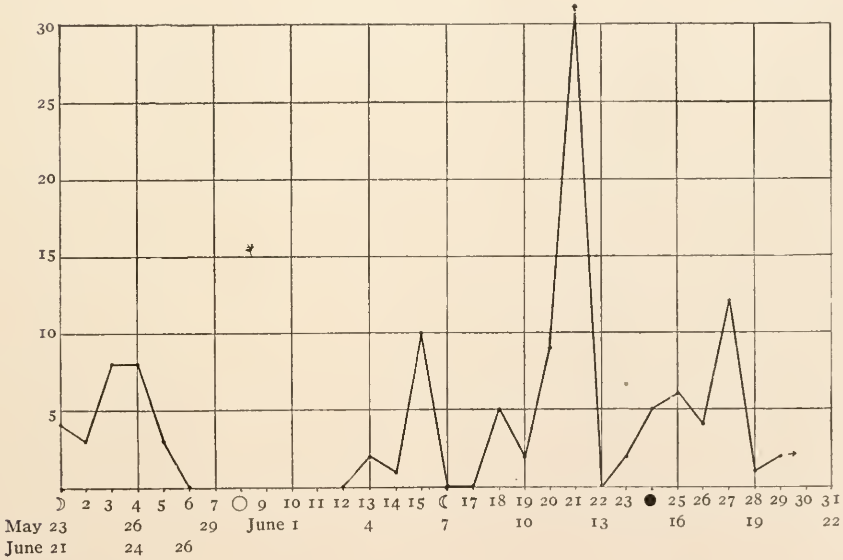
CURVE 5. The September run, 1911.

CURVE 6. The June run, 1912. The part of the curve to the left is the end of the June run as indicated by the arrow at the extreme right, and by the second row of dates which refer to this part of the curve (see footnote, p. 154).

CURVE 5

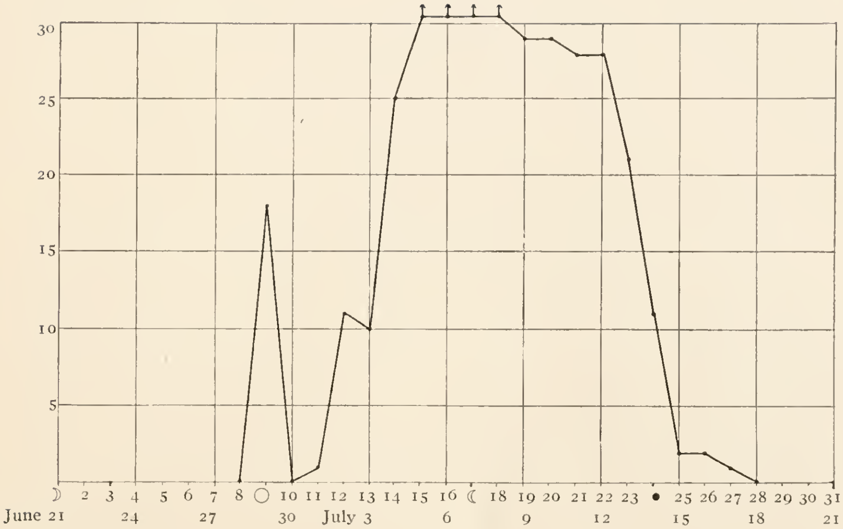


CURVE 6

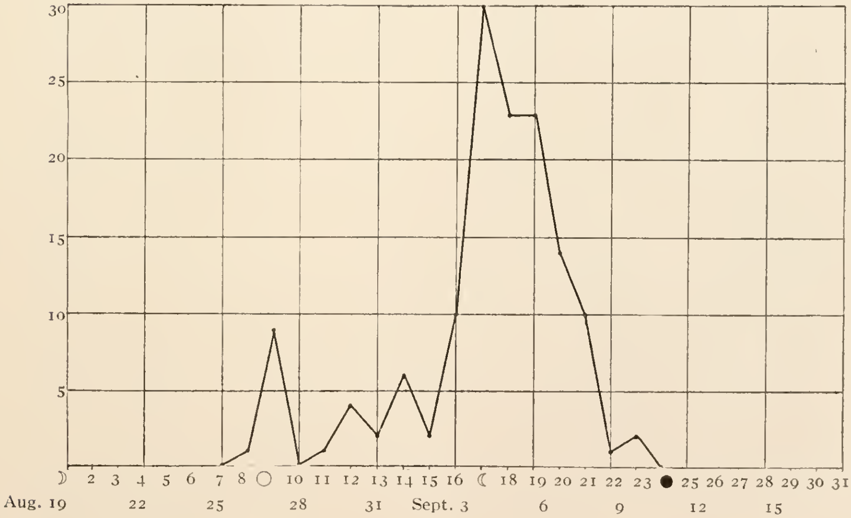


Curve 7. The July run, 1912.
Curve 8. The September run, 1912.

CURVE 7



CURVE 8



THE EFFECT OF DISTILLED WATER UPON THE FIDDLER CRAB.[1]

J. F. ABBOTT.

At present, *Fundulus* offers the only exception[2] to the statement that the unprotected epithelial surfaces of marine and fresh-water animals are permeable to distilled water and to electrolytes in solution. In all cases life appears to be preserved by some sort of an "adaptation" as yet unexplained which limits the permeability or controls it so as to maintain the integrity of the body fluids. Sumner[3] has shown that the gills in a variety of Teleosts are permeable, and Scott and White[4] have conclusively shown the same thing in *Mustelus*, while Garrey[5] and others have demonstrated the fact in a great number of invertebrates. The apparent exception offered by *Fundulus* is therefore the more remarkable.

While working at the Beaufort Laboratory of the U. S. Fisheries Bureau my friend Professor W. E. Garrey called my attention to the fact that the fiddler crabs (*Uca pugillator*) which abound in that region were passing indifferently from the sea to the overflow of an artesian well and he suggested that this form, like *Fundulus*, might offer an exception to the statement just made regarding the permeability of membranes. A few tests seemed to confirm this, for the crabs appeared to live indefinitely in pure distilled water as well as in salt solutions of various osmotic densities. The writer therefore devoted the greater part of the past summer to a study of the reactions of this animal to various salt solutions. For the privilege of working at the Beaufort Laboratory he is indebted to the kindness of the Hon. George M. Bowers, U. S. Commissioner of Fisheries, and to Mr. Lewis Radcliffe, the director of the laboratory, he is under especial obligation for the constant courtesy and generous assistance of which he was at all times the recipient. The present paper deals only with the effect of pure distilled water upon the fiddler crab.

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[1] From the Laboratory of the U. S. Bureau of Fisheries at Beaufort and the Zoölogical Laboratory of Washington University.

[2] Species of *Cyprinodon* and *Gambusia* apparently behave in the same way, although the facts have not been thoroughly worked out.

[3] U. S. B. F. Bull., 1905, Vol. 25, p. 97.

[4] *Science*, N. S., 1910, Vol. 32, p. 767.

[5] *Biol. Bull.*, 1905, Vol. 8, p. 257.

The crabs inhabit burrows in the sandy or marshy shores, the openings of which are usually, though not always, covered by the tide. At high tide the crabs retreat to their holes which they plug up with sand. As the tide ebbs they emerge and begin feeding, frequently going a considerable distance from their holes. In the interval between high tides, if the sun is bright and the day hot the males go through characteristic and energetic "dances." Altogether they spend many hours of vigorous activity out of the water. A related genus of the same family, *Ocypoda*, lives out of the water almost entirely and spends most of its time running about on the dry sand.[1] In accordance with this habit a peculiar modification has developed in connection with the gill chambers. In these two genera these are very large and cavernous, the two chambers together equalling approximately the space occupied by the viscera. The gills lie in the floor and the large space above them is normally filled with liquid. Communicating with this chamber, in addition to the ordinary openings for the exit and entrance of water, there is an aperture between the basal joints of the third and fourth legs, bounded by chitinized lips which bear tufts of hairs. This opening communicates between the cavity above the gills and the exterior by a sort of canal provided with a ciliated valve. By means of this arrangement, not only is the animal able to store up a large amount of water above the gills but it is also possible for a constant gaseous exchange to take place between this contained water and the air without. In other words the crab when out of the water is able to breathe air.

This accounts for the apparent tolerance of the animal to pure distilled water. When immersed in distilled water it feeds out through this channel some of the sea water contained in its gill chambers. As with some other crustacea a very small amount of NaCl in the surrounding medium is sufficient to preserve the integrity of the membranes and permit the animal to live in spite of the greatly altered osmotic pressure. That the crab accommodates itself in this way was shown by the following experiment. Five crabs that had been running about in a dry tank for a week were rinsed off in running fresh water, dried, and immersed in 250 c.c. of pure distilled water which was removed and renewed at short intervals. These samples were then titrated for chlorides by the Volhard method with the following results:[1]

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[1] Cf. Cowles. Carnegie Inst. Papers, Tortugas Lab., II., 1908.

Interval of Immersion in Pure Distilled Water.	Cl in Milligrams.
30 minutes	0.038
1 hour	0.188
2 hours	0.263
3 hours	0.132
3½ hours	0.297
Total Cl	0.918

The crabs were moribund in another half hour. The gill chamber capacity of each crab was roughly ½ c.c. The percentage of Cl in Beaufort sea water is 1.963 gm. per 100 or .049 gm. to 2½ c.c. The detectible salinity emitted by the crabs was thus only about 20 per cent. of that of sea water. Some may have been "held back," but it is more probable not only that the crabs in the experiment, which had been out of water for a week, but also crabs running about out of the water normally have their gills bathed by a liquid of only about one fifth the concentration of sea water. Experiment has shown that they will live indefinitely when immersed in very much greater dilutions, although I have not found that they will endure such a dilution as that used by Bullot[2] for fresh-water *Gammarus* ($0.00008N$).

In subsequent experiments the side of the gill chamber was cut away and the contents rinsed out with distilled water so that not only were all salts washed away but the gills were directly exposed to the action of the medium. Even under such circumstances the crab does not at once die when placed in distilled water but will live actively usually from four to six hours. Death appears to be due to a loss of necessary salts by diffusion out of the body fluids together with a disturbance of vital equilibrium on account of the absorption of water. In most marine invertebrates death very quickly follows a transfer into fresh water, that is, the limiting membranes are absolutely permeable to water and osmotic diffusion is very rapid. (The body fluids of all marine invertebrates, so far as known, are isosmotic with sea water.) In *Uca*, on the other hand, the gill membranes are apparently impermeable to water until the strongly solvent power of the distilled water has attacked or partially dissoved them and thus rendered them partially permeable. That they are eventually rendered permeable is shown in the following experiments. A larger species of *Uca* (*U. minax*) was chosen because of its greater size, but the habits and reactions of the two species are identical. The carapace of each crab was cut away on both sides to expose the gills, and the gill chamber was rinsed out. The carapace was then thoroughly dried with absorbent cotton and the crab was carefully weighed, and immersed in 200 c.c. of water distilled in Jena glass over potassium dichromate and sulphuric acid. At intervals this water was changed for a fresh amount and the sample titrated for chlorides with potassium thiocyanate against silver nitrate, using iron alum as an indicator. The following results were obtained:

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[1] For assistance in carrying out this portion of the experiments as well as for many helpful suggestions the writer is indebted to Mr. Wm. J. Crozier.

[2] Univ. of Calif. Pub. Physiol., 1904, Vol. I., p. 199.

Table I.

I.	II.	III.	IV.	V.	Chlorides (in Mg.) After
0.205					1 hour.
	0.135		7.420	5.312	3 hours.
5.600	9.431	26.09			5 hours.
9.210		1.700	(died)	0.954	7 hours.
					9 hours.
		8.91			11 hours.
15.016	9.516	36.705	(7.42)	6.266	Total chlorides.
7.588	3.544	8.463	6.011	4.638	Original wt. in grams.
7.813		8.460	6.599	4.833	Final weight.
0.225	0.677	0.005	0.588	0.195	Gain in wt.

Remarks.

Crab No. 1 died shortly after 7 hours.

Crab No. 2 died after 4 hrs., weighed immediately. Big claw cast at beginning, wound seared with nitric acid.

Crab No. 4 died shortly after 7th hr.; final chloride determination not taken.
Crab No. 5 died shortly after 7th hour.

It will be seen that without exception all the crabs gained in weight through immersion in distilled water. Likewise all the crabs lost chlorine, presumably through diffusion. There is no doubt that some of this loss may have been occasioned by bleeding, yet the outer shell of the gill chamber is very little vascular and such a loss must be inconsiderable. It, however, might be sufficient to account for the irregularities in the amounts of chlorine lost by the various crabs. An exception must be made of Crab No. 3 of which the branchiostegite was cut away very deeply and bled into the water. This accounts for the very small gain in weight and exceptionally high chlorine loss. Yet this individual for some reason not evident lived longer than any of the others.

If death is brought about by the extraction of necessary salts from the organism it ought to make considerable difference whether the amount of water that surrounds the animal is great or small. For we have seen that the presence of a very small amount of salt in the water (such as exists in ordinary well water) is sufficient to preserve the membrane or at least to greatly retard its solution by the water. If the amount of water were scant the crabs might be able, by losing a small amount of salts to it and thus raising the osmotic density of the medium ever so slightly, yet thereby to retard the solvent effect of the distilled water on the gill membranes. To test this, fifty tall, wide mouth bottles were arranged, in each ten of which was put, respectively, 50 c.c., 100 c.c., 150 c.c., 200 c.c., and 250 c.c. of distilled water. In each bottle was placed a crab (*U. pugillator*). The gill chambers were not opened but the lot were subjected to running fresh water for fifteen minutes, a procedure which experience has shown displaces practically all the salt water that may be retained in the gill chamber. The results are tabulated below, the figures representing the number of crabs alive at the different intervals of time.

TABLE II.

	50 c.c.	100 c.c.	150 c.c.	200 c.c.	250 c.c.
4 hours	10	10	10	10	10
8 hours	10	10	9	9	8
12 hours	10	10	8	6	2
18 hours	10	7	5	4	0
22 hours	10	1	1	0	
24 hours	9	1	0		
48 hours	6	0			
72 hours	2				

SUMMARY.

1. The fiddler crabs of the genus *Uca* are able to endure abrupt and profound changes in the osmotic density of the water which they enter, passing from sea water into ordinary "fresh" water with impunity.

2. They are able to spend long periods out of the water by the aid of a mechanism by means of which they store up a quantity of water above the gills and aerate it directly by contact with the air.

3. Their gill membranes are only relatively not absolutely impermeable. In the presence of pure distilled water they both lose salts (as indicated by the titratable chlorine) and also gain weight through the absorption of water.

4. They will live a much longer time in small quantities of distilled water than in larger quantities. This is probably due to the fact that the salts diffused out from the tissues although small in amount are sufficient, in a small quantity of water, to raise the osmotic tension of the latter sufficiently to inhibit its solvent action on the gill membranes and thus to retard the further diffusion of salts. In a larger quantity of water this point cannot be reached before the debilitating effect of the loss of salts results in the death of the crabs.

ON THE ORIGIN OF DOUBLE-YOLKED EGGS.

OTTO GLASER.

INTRODUCTION.

By comparing five cases of double eggs that happened to fall into his hands, with a considerable number described in the literature, Parker ('06) was able to divide these abnormalities, on the basis of the factors probably involved in their production, into three classes: "First, those whose yolks have come from an abnormal ovary, but have passed through a normal oviduct; secondly, those whose yolks have come from a normal ovary, but have passed through an abnormal oviduct; and finally those produced by an ovary and oviduct, both of which have been abnormal in their action" (p. 17).

To the first group belong eggs which contain ordinarily two yolks, surrounded either by individual vitelline membranes or by a common one. The second class is made up of eggs in which normal yolks are imbedded in abnormal secondary envelopes, whereas the third is composed of cases in which one egg, consisting usually of shell, shell-membranes, albumen and a small yolk, is enclosed in an outer one of normal construction. Nine cases found in the literature by Parker, four additional ones studied by himself, as well as some of those referred to by Hargitt in a neglected paper ('99) and in a later one ('12), belong to this group.

In this article I shall report observations on a case belonging to the first class, and I do this, not because double-yolked eggs are sufficiently rare to warrant further description, but because I have been unable to find in the literature accessible to me any account of the ovarian abnormalities associated with the production of this type of egg. In fact from Parker (*loc. cit.*, p. 16) one gains the impression that these peculiarities are hardly marked enough to justify the use of the word "abnormal." Thus he says: "The laying of eggs with two yolks may become, as Landois ('78, p. 24) declares, almost habitual with certain hens. Bartels ('95, p. 143) states that the hen that laid the double egg described by him had often laid such eggs, and Immermann ('99, p. 8) records the case of a hen that laid such an egg about every eight days. Apparently this is as much an organic peculiarity of certain hens as is the production of twins by certain individuals in the human species, and while it may be called abnormal in that it is unusual, it is in no sense indicative of serious organic derangement or disease."

Despite the fact that the origin of double-yolked eggs can be attributed, as it is by Parker, to an ovarian peculiarity, such as the simultaneous discharge of two yolks from separate follicles, or the rupture of one follicle containing two yolks, there nevertheless are two other possibilities. In the first place, an ovum discharged into the infundibulum might fail to be moved downward by peristalsis until the ovary had discharged a second time, in which case we might be dealing either with a deficiency of substances normally inducing these movements, or with subnormal irritability on the part of the oviduct. In the second place, the "organic peculiarity of certain hens" may have an ovarian basis decidedly "indicative of serious organic derangement" if not of disease. The ovary which I have studied is certainly pathological although I cannot conclude that the conditions found are the only ones that induce double-yolked eggs, or that they always do so.

Unfortunately the bird on which my data are based died during my absence and the oviduct was not preserved. However the ovary seems to me capable of explaining why she laid abnormal eggs, although this does not show that the oviduct was normal either in structure or in action. Normality however seems likely, for if there had been peristaltic difficulties it seems reasonable to suppose that occasionally at least an egg abnormal in other respects would have been produced. This is not known to have happened in the five years during which this hen was under fairly constant supervision.

Clinical History of the Individual.

The bird in question, a white leghorn belonging to Mrs. Wm. Looker, of Ann Arbor, was hatched in the spring of 1906 and lived until June, 1911. She was a beautiful example of the breed, but had an unusually large comb. She was a loud and boisterous cackler, and always made a great fuss after laying. Apparently she never exhibited any tendency to set. Although it cannot be stated with certainty that she never laid normal eggs, it is certain that a very large percentage was abnormal.

The eggs during her last year measured on an average 7.5 cm. in their long diameter, 4.5 cm. in their short. The yolks, always in individual vitelline membranes, were equal in size with an average diameter of 3 cm. They were always in contact, or practically so, and were surrounded by a common jelly mass.

The records are too meager to show whether there was any rhythm in her laying. On occasion she laid double-yolked eggs on two or even three successive days. Spells of hyperactivity such as these were frequently followed by periods of indolence lasting from one to several weeks. Sometimes she would lay every other day for a period.

Although the eggs were always large they decreased in later years. Even then laying caused difficulties as evidenced by noises suggestive of struggle. After a period of laying the bird nearly always "moped," hanging her head and refusing to eat. These sick spells were not noticed during the first year or two, although there was never any eagerness for food.

I have given these details because they may be valuable as clinical symptoms, and because they show that the ovary, which, as we shall see, was abnormal structurally, was so physiologically as well. In fact I am inclined to think that the structural peculiarities of this organ are a consequence primarily of some physiological defect, and that the abnormal spatial relations brought about in this way gave the physical basis for the production of double-yolked eggs.

THE ANATOMY OF THE OVARY.

The ovary was removed immediately after the death of the bird and fixed in Zenker's fluid by my brother, R. W. Glaser. The gross appearance as well as numerous details are reproduced in Fig. 1, which I made by first printing an outline of the organ through glass on blue-print paper, and after transferring this to bristle board, filling in the necessary minutiæ freehand.

Even a casual glance shows that this organ is markedly abnormal. Most striking of all is the presence of many medium-sized follicles which, instead of being held to the main mass of the ovary by relatively short, stout necks of tissue, are attached

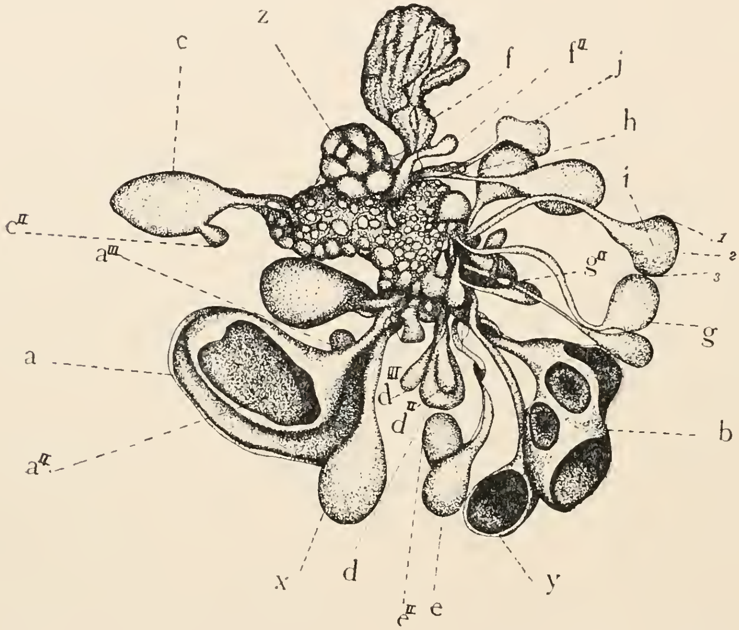

FIG. 1. Abnormal ovary of hen that laid double-yolked eggs. Description in text, p. 178.

by suspensoria more or less twisted and at times over 3.5 cm. in length. Originally these appendages formed a complicated snarl. To facilitate both drawing and description they were carefully dissected apart as in the figure.

Four other features, some of them equally striking, are worthy of note. First are the compound follicles, *a* and *b;* second, apparently budding follicles at *c*, *d*, and *e;* third, apparently branching suspensoria, *f*, *g*, and *h;* and finally a considerable number of fine threads running at various angles from one suspensorium, follicle, or portion of the central mass, to another.

The origin of these various structures can be inferred with reasonable certainty in most cases. As far as the nature of the suspensoria is concerned, I think there can be little doubt, although there are at least two possibilities as to the mechanism of their development. Sections show that they are composed entirely of connective tissue, and that they may or may not possess a lumen. Blood vessels are abundant. From these facts, as well as from the total absence of granulosa cells, I conclude that the suspensoria are not modified follicles, but greatly elongated projections of the ovarian surface, tunica albuginea, filled with stroma.[1]

I am not certain how these elongations have arisen. It is conceivable that they are the products of abnormal growth; it is equally conceivable that they are the result of stretching, due to weakness or readiness to flow on the part of the tunica albuginea. In a bird in its normal upright position, the sudden development (Riddle, '11) of a large yolk mass in a given follicle must seriously increase the load sustained by the tunica, and if this for any reason is weak, stretching in these regions might easily result. It is interesting in this connection that all of the follicles with long suspensoria are either well along in their growth periods, or are attached to those that are. Comparative study of the compound follicles, of the branching suspensoria, and of the threads makes me incline definitely toward the second hypothesis.

Such study not only suggests that all the structures mentioned are the outcome of one and the same process, but also how the more complicated follicles may have originated. *B* is a good one to begin with. Before dissection the surface of this irregular follicle gave but faint indication of the four completely separate cavities which it contains. Sections of the interfollicular walls show one continuous connective tissue mass bridging the distance from one granulosa to the other. This latter membrane in all the follicles sectioned appears much hypertrophied. It is at least four times thicker than normal and contains quite large intercellular spaces or lacunæ.

How such a complex follicle could have originated can be answered by studying the others. Follicle *c* suggests that compound follicles might have originated by a process of budding. To test this view part of the wall of *c* together with the attached follicle c'' was cut into sections 10μ thick. To my surprise no connection whatever between the lumina of the two follicles could be found. The place where this was expected showed the granulosa and fibrosa of both, and between the fibrosæ, a thick connective tissue mass highly vascularized. I examined other apparently branching follicles, such as *e*, and again found the same thing true. In certain other instances connections between adjacent follicles do exist, but these are not via the necks of the attached follicles, but through their sides, and must therefore have come about secondarily by the disappearance in these places not only of the inter-follicular tissue, but also of the granulosæ and fibrosæ. If c'' then is not a bud, how did it come to occupy its present position?

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[1] This naturally suggests the question whether the enlargements at their distal ends are really follicles, or merely abnormal structures superficially resembling them. The discovery of large yolks with smooth surfaces within these bodies, as well as the presence of fibrosæ and granulosæ all in proper relation, leaves no doubt that we are dealing with genuine egg follicles.

In *d*, follicles d'' and d''' are attached to the main suspensorium by short necks of their own. These however are so completely fused proximally with the suspensorium of *d* that their independence is largely obliterated. E'', although firmly fused to *e*, really has a suspensorium of its own, but this is reduced to an extremely fine thread, visible as an independent attachment only in one or two places. C'' upon careful examination proves also to have indications of an independent attachment to the central ovarian mass. The threads therefore are reduced suspensoria, and the compound follicles are fusion products.

The question how these fusions occur remains to be answered. Indications as to how these could have come about are contained in the facts just discussed, for these suggest that one follicle is carried away from the central ovarian mass by another. If this is true, we should be able to discover various stages in the process. Accordingly we can interpret *b* as a late stage in which intimate union has occurred among the follicles distally, whereas proximally the suspensorium of the group shows the multiple nature of its origin by its distinct division into a number of strands. *A* is another late stage, only here the follicles seen are larger than in *b*, their fusion is less complete, and a'' has worked its way into *a* so that the yolk of the latter came to lie between the granulosa of the outer follicle and the connective tissue covering of the inner one. In this case, too, dissection clearly exposes the compound nature of the suspensorium. *C*, *d*, and *e* represent stages of fusion less complete than the preceding.

If the suggestions made by these follicles and suspensoria are really correct it ought to be possible to find small follicles not only in places where they are not obvious, but also at various levels of the suspensoria, and this has actually been done. For instance, *a*, prior to dissection, was not obviously double. *J* is not simple and somewhat distorted, but double, one follicle being considerably larger than the other; *i* is triple, having a central follicle, 2, and two equal-sized smaller ones, 1 and 3. The one on the right, 3, communicates with 2 by means of a small irregular aperture. No opening connects 1 and 2. In both *i* and *j* the suspensoria appear to be united, and there seems no way of explaining how the extra follicles got into their present position, than by assuming that the same process brought them there that carried the main follicle away from the ovary.

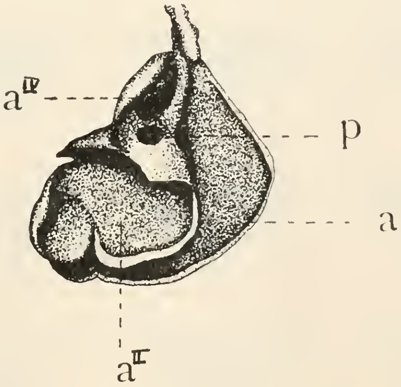

Fig. 2. Dissection of compound follicle, a, a'', and a''', Fig. 1. Natural size. In this figure a''' is omitted. The suspensorium of a^{II} has been opened and an additional follicle a^{IV} is exposed. The pore, p, establishes a communication between the follicular cavity of a^{II} and a^{IV}.

Most illuminating in this connection is the presence of accessory follicles on the suspensoria. Sometimes these are large enough to be easily seen, as in the case of a''', of f'', attached to the neck of *f*, a large empty double follicle, and of g''; occasionally they are quite small, like those on the necks of *c* and *j*, whereas at times they may be completely hidden, as in the neck of *a''*, where another follicle was discovered only after the dissection shown in Fig. 2 had been made.

Given weakness on the part of the tunica albuginea, three minor variations of one and the same process are capable of accounting for these different types of follicles. I have tried to represent this in a series of diagrams (Fig. 3).

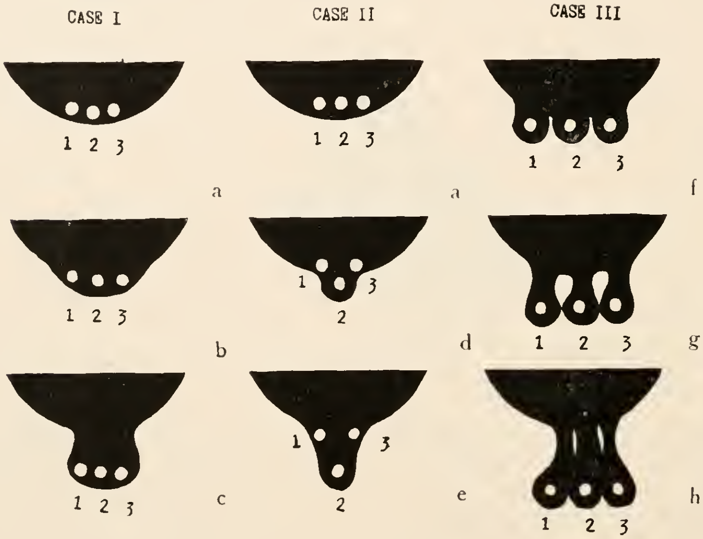

FIG. 3. Diagrams to illustrate the origin of compound follicles. Description in text, p. 182–183.

If we imagine that the ovarian surface evaginates rather broadly beneath egg 2, Case I., diagram *a*, then eggs 1 and 3, which are supposed to lie very close to 2, will be carried away from the ovary, as in *b* and *c*. This would lead to the formation of a follicle of the type *i* and *j*, Fig. 1.

If on the other hand only a very small evagination should take place under egg 2, Case II., its neighbors, 1 and 3, if carried away from the ovary, would not come to lie in such close proximity to 1 in the evagination. In fact they would lie higher up in the suspensorium, *d*, *e*, Fig. 3, and in this manner sufficiently great differences in early distribution would account for the later development of follicles at various points on the suspensoria as in *a*, *c*, *f*, *j*, etc., Fig. 1.

If, owing to greater distances between the eggs, or to greater stiffness on the part of the tunica, separate evaginations should occur beneath three follicles as in Case III., diagram *f*, Fig. 3, these if not too remote from one another might give rise to a triple follicle, having a common suspensorium except near the ovary where its multiple origin would still be apparent upon close examination. These processes, represented diagrammatically in *g* and *h*, would give rise to complexes similar to *a* and *b*, Fig. 1. If the number of follicles involved were greater, more complicated results would follow. The possibilities of greater complication are clearly indicated by a group of relatively young follicles such as *z*, Fig. 1, as well as by instances in which follicles, of patently distinct origin proximally, have fused distally with others with whom they were brought into contact secondarily by mere accident. Good examples of adhesions of this kind are follicles *x* and *y*, Fig. 1.

In its relation to the production of double-yolked eggs, this ovary is full of suggestions. Follicle *f*, Fig. 1, is an empty double one, and there can be little doubt that it was instrumental in the production of one of the abnormal eggs. Follicles *a* and a'' contained yolks of essentially the same size, and there seems to be no good reason for doubting that these two would have been shed at the same time. In *b*, the follicles fall into two groups of two each. In one of these they are distinctly larger than in the other, but the follicles within each group are of the same size. It seems likely that in this complex we have the physical basis for the production of two double-yolked eggs in rapid succession. *Z* suggests the possibility of a series of abnormal eggs such as this bird is known to have laid on several occasions.

Physiology of the Ovary.

The morphological findings and their interpretation leave open the question as to how one may picture the physiology of this organ. Follicle *b*, Fig. 1, demonstrates the possibility of relatively independent growth on the part of two groups of eggs, as well as the possibility of synchronism in eggs belonging to the same group. How this can be is answerable by assuming that the vascular supply to the two groups was different, whereas to the individual members of each group it was practically the same. The physical basis for this might easily be found in the character of the fusion undergone by the individual suspensoria, for if this should be such as to partially occlude the flow of blood in certain directions, the follicles supplied by the vessels involved would lag behind those furnished with a better supply. That the blood supply to certain follicles must be affected, is clearly shown by the suspensoria which are reduced to mere threads.

In certain instances, however, a follicle despite its reduced suspensorium is practically as large as the neighbor to which it is attached, as in the case of e''. This suggests the establishment of a secondary blood supply derived from the better equipped follicle. The highly vascular condition of the connective tissue between the granulosæ certainly is favorable to this interpretation. In fact in view of the structural relations it is difficult to see how the blood supply could be otherwise than identical in this case.

Identity of vascularization makes another fact understandable. A follicle such as c'' would under normal conditions have a blood supply much less than that of its "host" *c*. As it probably is dependent on that of *c*, however, owing to its intimate fusion with it, and the reduction to almost nothing of its own suspensorium, it is likely to have more material brought to it than it can metabolize in normal fashion. This follicle, as well as others of its size similarly placed, showed considerable depositions of yolk in the lacunæ of their hypertrophied granulosæ.

This fact is not only suggestive as to the rôle played by the granulosa during oögenesis, but also of the part of the egg itself in initiating the deposition of yolk, for if the ovum in this instance is not supposed to have been less permeable to the raw materials of yolk than the one in the larger follicle, where only traces of yolk could be found in the granulosa, it is difficult to see why the two eggs should have been unequal in size. There must be a period in the ovarian life of an egg when its permeability to certain substances is suddenly greatly increased. Riddle's work ('11) suggests the same thought, but this abnormal ovary indicates that the conditions followed by increased permeability are to be looked for in the egg. If ova in the same state have an identical blood supply they will undergo equivalent growth, but mere identity in their circulation does not insure this.

Although these considerations are necessarily speculative, their possible relation to more remote matters may be briefly mentioned. It is certainly a remarkable fact that in the human ovary, to mention a case among mammals, and in the hen's ovary, to mention one among birds, normally one egg ripens after another, whereas in the ovaries of amphibians and fishes thousands of eggs may ripen at one time. It is quite possible that the conditions which in the one case lead to an abnormality are identical with those which in the other give a typical result. In no case do all the eggs contained in any of these ovaries ripen at the same time. This certainly points to alterations in the permeability of the ovum as a factor in the initiation of its growth period, and suggests that these changes are directly traceable to the activity of the egg. The fusion of normally distinct follicles in the hen's ovary brings about secondarily an identical vascularization and thus accidentally duplicates the nutritive conditions prevalent in the ovaries of certain other forms.

Identity of blood supply then is suggested as an explanation of the synchronic yolk formation in these eggs, whereas a change in permeability undergone independently on the part of the eggs themselves introduces this period of growth. This change in permeability must necessarily also be synchronic if double-yolked eggs are to be produced, but how this synchronism is brought about in certain eggs, and how it is prevented in others, cannot be profitably discussed in our present state of knowledge.

The important thing after all is that a bird's ovary, with follicles secondarily fused as described in the preceding pages, does actually give rise to a series of double-yolked eggs, and this remains true even if my suggestions as to the development and physiology of this organ should prove to be entirely wrong. It is to be hoped that the clinical symptoms given may aid in the discovery of further material upon which more detailed studies in the living state could be carried out with great advantage to our knowledge of the more obscure points in the physiology of egg-production.

Zoölogical Laboratory,
University of Michigan,
November 22, 1912.

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PRELIMINARY NOTE ON THE RESULTS OF CROSSING TWO HEMIPTEROUS SPECIES WITH REFERENCE TO THE INHERITANCE OF AN EXCLUSIVELY MALE CHARACTER AND ITS BEARING ON MODERN CHROMOSOME THEORIES.

KATHARINE FOOT AND E. C. STROBELL.

Interest in the studies of the chromosomes has been greatly stimulated during the past few years by the revival of the hypothesis that they are the bearers and distributors of all hereditary characters. Belief in such a fundamental significance of the chromosomes has led its firmest adherents to interpret every phase of their morphological and physiological expressions into terms of a causal nature, even to the point of claiming that definite chromosomes—such as the so-called sex-chromosomes—are the determining factors of sex and of all sex-linked characters.

On the other hand a large number of cytologists have studied the chromosomes from an entirely different point of view—believing that their morphological phases are not to be interpreted in terms of a causal nature, but like many other organs of the cell, they are the expression rather than the cause of cell activities.

These opposing interpretations can be strikingly demonstrated by a few quotations from recent papers. Morgan ('11) writes: "The experiments on *Drosophila* have led me to two principal conclusions: First, that sex-limited inheritance is explicable on the assumption that one of the material factors of a sex-limited character is carried by the same chromosomes that carry the material factor for femaleness.

"Second, that the 'association' of certain characters in inheritance is due to the proximity in the chromosomes of the chemical substances (factors) that are essential for the production of those characters" (pp. 365).

Wilson ('12) gives the stamp of his approval to Morgan's conclusions. He says Morgan's results "bring strong support to the view that the chromosomes are such bearers of unit-factors, for the whole series of phenomena determined in *Drosophila*, complicated as they seem, become at once intelligible under the assumption that certain factors necessary for the production of the sex-limited characters are borne by the *X* chromosome; and without this assumption they are wholly mysterious" (pp. 420–1).

As opposed to these definite expressions of faith in the causal nature of the chromosomes, we may quote Child's latest repudiation of all such hypotheses. "Let us take the case of the chromosome, for example, which plays so important a part in recent biological hypothesis. What is the chromosome? If it is what many authors seem to believe, it is an autonomous being endowed with something more than human intelligence. But if we are not willing to believe this, then we must regard the chromosome as an incident or result of dynamic processes in the organism, like other morphological entities. If this is the correct view, then it is nothing ultimate or fundamental. We must analyze it into terms of the processes which have made it, and in this analysis we shall sooner or later find nothing more or less than the whole complex of processes which constitute the organism. The organism makes the chromosomes, not the chromosomes the organism" (pp. 33).

Our cytological studies have caused us to sympathize with the many investigators who have expressed skepticism of the causal nature of the chromosomes. For several years we have argued that the chromosomes in the forms we have studied show too much variability, both in their morphological and physiological expressions, to justify those theories which obviously demand a rigid compliance to a definite mode of expression. We demonstrated in 1905 that the form and relative size of the chromosomes in *Allolobophora fœtida* are inconstant and in every publication since that date we have demonstrated variability in the form, relative size and behavior of the chromosomes in every form we have studied, and we have consistently argued that such variability attacks the very foundations upon which the popular chromosome speculations of this decade have been built.

Recent experimental results have caused a marked modification of the views of some of the adherents of the more extreme chromosome hypotheses, forcing them to modify the theory of the individuality and continuity of the chromosomes so far as to admit that there must be an interchange of chromatin between individual chromosomes and that the chromosomes which emerge from synapsis are "probably not identical with the original conjugants" (Wilson, '12, pp. 422). They do not however extend this interpretation to the *XY* chromosomes: "the degree of union may vary in different cases, involving sometimes no fusion, as is suggested by the history of the *XY* pair" (Wilson, '12, pp. 417). This would seem to be an inevitable conclusion, otherwise any facts that could be assumed to be explained on the supposition of an interchange of factors between *X* and *Y*, would be inexplicable for those forms in which no *Y* is present.

In analyzing the results of our recent experiments we shall accept, for the sake of the argument, the above assumption that there is no interchange of material between the *XY* chromosomes, and also the hypothesis of male- and female-producing spermatozoa.

In 1909 Castle suggested that the *Y* chromosome of those forms in which this morphological element is present may be the bearer of all characters that are exclusively male. He wrote: "I would offer the suggestion that we have a mechanism suitable for the transmission of characters exclusively male in the *Y* element described by Wilson, the 'synaptic mate' of the *X* element."

It would seem possible to test the value of this suggestion by crossing two species, each having the *XY* chromosomes, and one of the species having an exclusively male character which is lacking in the other. These conditions are met in the two Hemipterous species *Euschistus variolarius* and *Euschistus servus*. The former has a character that is exclusively male, in the form of a distinct black spot on the male genital segment, while such a spot is entirely lacking on the male genital segment of *Euschistus servus* (Photo 1). There is no black spot on the female genital segment of either species.

During 1911 and 1912 we succeeded in crossing three species of Hemiptera, but in this preliminary note we shall confine ourselves to discussing the cross between *Euschistus variolarius* female and *Euschistus servus* male. From this cross we were able to raise to maturity 11 males and 16 females of the F_1 generation and these fortunately mated readily and proved to be very fertile. Seven of these pairs were isolated and the offspring of each pair raised in separate cages—in many cases even the bugs from a single batch of eggs being isolated during the entire period of their development. From these seven pairs of the F_1 hybrids we raised 249 females and 204 males. These bugs were kept in the laboratory until several days after they had reached the winged stage, when the females were preserved as pinned specimens and 191 of the males were preserved in glycerine, as shown in photos 2[1] to 6.

An analysis of the maturation divisions would seem to support Castle's suggestion that the *Y* chromosome may be the bearer of exclusively male characters, for this chromosome is the only one that can be in both the so-called male-producing spermatozoa of each quartette of spermatids resulting from the two maturation divisions, assuming that these divisions occur as illustrated in the text-figure.

It would seem quite logical to conclude that if a character associated exclusively with the male sex can be inherited from the father, the factors which produce it should be in the so-called male-producing spermatozoön and if they are to be located in a given chromosome it must be the one and only chromosome that *ex hypothese* can be present in all the so-called male producing spermatozoa, otherwise the character would not be a constant feature.

If Castle's suggestion is correct, then all characters exclusively male can be inherited only through the father, as the *Y* chromosome is never in the female. If then *Euschistus variolarius* is fertilized by *Euschistus servus*—a form which lacks the spot—none of the offspring should have it, neither the F_1 nor the F_2

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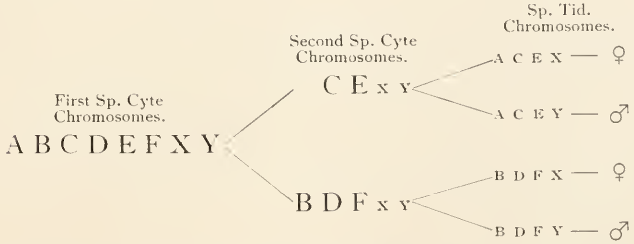
[1] We have photographs of all the male hybrids, but they must be reproduced by the bromide print method in order to secure an accurate reproduction of the shades of difference in the size and intensity of the spot. We hope to be able to reproduce all these photographs when we publish the detailed account of the crossing of the three species, *Euschistus variolarius*, *Euschistus servus* and *Euschistus ictericus*.

generation. *As a matter of fact, however, the spot of Euschistus variolarius is transmitted through the female—appearing in a slight degree in the males of the F_1 generation and much more intensely in the F_2 generation*—some of the offspring of this generation having the genital spot quite as conspicuous as that of their maternal male ancestors—*E. variolarius.* Such facts would seem to dispose beyond question of Castle's suggestion that the factors which produce exclusively male characters must be located in the *Y* chromosome.



Scheme of the two maturation divisions of *Euschistus variolarius* and *Euschistus servus* based on the assumption that the first maturation division separates autosomes of maternal and paternal origin and the second division halves them. The *XY* chromosomes on the contrary being halved in the first division and separated in the second division. The relative positions of the autosomes may be changed unless definite chromosomes are always destined to the same pole, but reversing their position in this regard does not alter the end result—that the only chromosome common to both so-called male-producing spermatids is the *Y* chromosome.[1]

We may next examine the facts on the basis of the assumption that the factors which produce the male genital spot can be located in the *X* chromosomes. If this is true then the spot cannot be transmitted through the so-called male-producing spermatozoön for this spermatozoön contains no *X* chromosome.

We hoped to be able to test this by fertilizing a pure female *E. servus* by a pure *E. variolarius*, but owing to great scarcity of material in the locality where we were working we were unable to find any male *variolarius* at the time it was possible to attempt this experiment. We were, however, able to cross a pure *E. variolarius* male with an F_1 female and compare the male offspring with those obtained by the F_1 matings.

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[1] We have used the method of designating univalents by the letters of the alphabet, bivalents being represented by A B, C D, E F. Only six of the twelve autosomes are designated.

If, as stated above, we attempt to locate the spot factors in the X chromosomes, we cannot expect these factors to be transmitted through the male producing spermatozoon and therefore if an F_1 female hybrid is fertilized by a pure *E. variolarius*, we should expect to have a no more pronounced spot in the offspring than we obtain by matings of two F_1 hybrids. *The facts, however, demonstrate that the spot is transmitted through the pure male E. variolarius far more intensely than it is through the F_1 male.*

This is strikingly illustrated by a comparison of the male offspring of an F_1 female and pure male *variolarius* with the 191 males we have succeeded in raising from seven pairs of the F_1 hybrids. We have 18 males from the former pair and many of them have the genital spot stronger than any of the 191 males from the F_1 parents. Further, none of these 18 males are without any spot, *whereas 84 of the 191 males of the F_2 generation have no spot whatever.*

Our facts seem to have demonstrated two points: First, that the spot is inherited through the female without the aid of the Y chromosome, and second, that the spot is inherited through the male without the aid of an X chromosome. We seem forced then to admit that in this case neither of the so-called sex chromosomes is necessary to the inheritance of an exclusively male character, the factors of which, it would seem, ought to be contained in a chromosome which determines sex, if, in fact, sex determining chromosomes exist. The spot in *E. variolarius* is a part of the male genital segment and it is only logical to assume that the factors which produce it are associated with the factors which produce the male genital segment itself, wherever or whatever those factors are. As the facts do not seem to warrant the assumption that these factors are confined to either of the so-called sex-chromosomes, we may re-examine them in the light of placing these factors in some other chromosome or chromosomes.

As the so-called male-producing spermatozoön of many forms has no Y chromosome and therefore no male sex-chromosome, it is obvious that if this spermatozoön is assumed to carry a factor which produces sex, this factor must be placed in some other chromosome, by those who believe that the chromosomes are the vehicles of such factors. Realizing this Morgan ('11) concluded that "the factors for producing the male must be located in some other chromosome" and he places these factors in a pair of homologous chromosomes, as shown by his scheme.

Gametes of female — X M—X M
Gametes of male -- X M—M.

A glance at text-fig. 1 will show that placing the male factors in an homologous pair of chromosomes (in *A* and *B* of Fig. 1 for example) necessitates their presence in all the spermatozoa of both sexes, thus giving to the so-called female-producing spermatozoön the male-producing factors in addition to the female-producing factors. The female-producing spermatozoön is therefore heterozygous for sex and the male-producing spermatozoön homozygous for sex—and this seems to involve a denial of the assumption that has been the basis of so much experimental analysis, namely, that the female is homozygous for sex. How can the female be homozygous for sex when the so-called female-producing spermatozoön has conveyed to her "the factors for producing the male"?

If we assume with Morgan that both members of a definite diploid pair of chromosomes contain the factors for producing a male, this must hold for the same diploid pair in both the female and the male, and if the factors producing exclusively male characters are linked with the factors for maleness, then each member of this diploid pair should contain the factors for producing the spot, which in *E. variolarius* is an exclusively male character.

As this diploid pair is in the female as well as in the male, it would seem necessary to assume that in *E. variolarius* the female carries an inhibitor of such exclusively male characters. But one is embarrassed in considering where to place this inhibitor—if we locate it in both members of an homologous pair of chromosomes, then it would be in the male as well as in the female. If we place it in only one member of an homologous pair (in *A*, for example, of text-fig. 1), this would involve selective fertilization, for if the female is fertilized by the so-called female-producing spermatozoön that is without the *A*, and she has by chance discarded the other A in her polar body, she also would have the spot, and further, half the male producing spermatozoa have the A chromosome and therefore they must not function or the spot would be absent in half the variolarius males.

If we place the inhibitor in the three X chromosomes, the assumption obviously would not work, for the male has one of the three X chromosomes and the spot would therefore be inhibited in the male as well as in the female. It would therefore be necessary to assume that in *E. variolarius* two doses of inhibitor are necessary to cancel the spot. This assumption, which apparently would hold for *E. variolarius*, is, however, found to be untenable if used to explain the results of the cross between *E. variolarius* and *E. servus*, for the female hybrid cannot receive a double dose unless we assume that *E. servus* carries an inhibitor for inhibiting in the female a male character which is never present in the male. If, however, we feel justified in assuming that the female hybrid has a double dose of inhibitor, this would fail to explain the absence of the spot in those male hybrids, which do not have it, for they have only one X chromosome and therefore only one dose of inhibitor. We might avoid this by assuming that one dose only would be necessary for the female hybrid, as she presumably has only one dose of spot factors; but this would involve inhibiting the spot in all the F_1 males also, which is opposed to the facts, as the spot is not inhibited in all the F_1 hybrids. The facts show that the spot factors—whatever they are—are latent in the female and if they are suppressed by an inhibiting factor, it would seem that this cannot logically be located in a definite chromosome.

Our results demonstrate that the spot is by no means a unit character. If we speak of it in terms of Mendelism we must say that it is the result of a large number of unit factors, for in the 107 males of the F_2 generation in which a spot can be identified, it is present in every degree of intensity, from a mere indication of a spot to those which are nearly, if not quite, as conspicuous as the spot of a pure *variolarius* (Photos 2–4).

If we assume that each member of one diploid pair of chromosomes contains all or half the factors for the genital spot, in addition to the factors for maleness, then the ripe egg of *E.*

variolarius contains one such chromosome and it follows when the egg is fertilized by *E. servus* it will have one diploid pair of chromosomes that is homologous for the factors producing a male, but heterozygous for the factors producing the spot.

Such a diploid pair should be present in all the offspring—both males and females, and if we cancel it in the females by assuming an inhibitor somewhere, we still have it in the males and therefore the spot should be quite as pronounced in the F_1 hybrid males as in *E. variolarius*. The facts, however, are as follows: None of the 11 male hybrids have a spot as strong as *E. variolarius*, 2 have no spot whatever, 3 have a faint spot—like that of the third bug from the bottom of the tube in photo 4—4 have a slightly stronger spot, and 2 have a spot about one-third as pronounced as that of *E. variolarius*. The genital spot in the F_1 hybrids is very variable, it is not expressed as a dominant, a recessive or a true blend.

If we attempt to explain these results in terms of chromosomal distribution of the factors, we encounter serious difficulties and are forced to assume the illogical position of suggesting a method of chromosome division for the hybrids which obviously does not occur in *E. variolarius*, as the spot is not variable in this form.

Assuming that the diploid pair of chromosomes in the egg of *E. variolarius*, which was fertilized by *E. servus* is heterozygous for the spot factors (that they are in chromosome *A*, for example, of the pair *AB*), then it must be asked why those in chromosome *A* are completely suppressed in some of the F_1 hybrids, while part of them find expression in other hybrids.

We might simply assume that some of the factors of chromosome *A* have dropped out, but the facts show this to be untenable for the spot reappears in the F_2 generation—in some cases quite as pronounced as in the pure *E. variolarius*. We must thus assume that the female *variolarius* has at least half the spot factors which she transmitted to the male hybrids, although these F_1 hybrids show either no spot at all or an incomplete spot. To account for these facts we must assume that the male hybrids differ from the pure males in having an inhibitor that inhibits those spot factors which are present but which are not expressed. We have seen that the facts will not allow placing the inhibiting factors in either the *X* chromosomes or the ordinary chromosomes and we are thus forced to admit that inhibiting factors—whatever they are—must be located outside the chromosomes—in the region of pure hypothesis. The facts force us to consign to these hypothetical inhibitors, not only the responsibility of suppressing the spot factors in all the females, but also of determining just how many spot factors shall find expression in the males of the F_1 and F_2 generations, and thus they practically relieve the chromosomes of the burden of unit distribution.

Chromosomes.—The small idiochromosome is an exclusively male character—a feature it has in common with the genital spot —and if it could be put to the same test of experimental breeding, which we have attempted for the genital spot—the results ought to show whether this idiochromosome has indeed the individuality which modern chromosome theories demand or whether—like the genital spot—its relative size is merely a structural feature which can be transmitted through the female. We confidently expected to be able to apply this test to the small idiochromosome of *variolarius*, for it had been demonstrated that this chromosome in *variolarius* was relatively much smaller than in *servus*.

The chromosomes of *Euschistus servus* were studied by Wilson in 1906 and he concluded that the *XY* chromosomes of this form differed from those of *E. variolarius* in their relative size. He says: "The above described species of *Euschistus*, while agreeing precisely in the general relations, present individual differences so marked as to show that even the species of a single genus may be distinguishable by the chromosome groups. In this case the most interesting feature is the series shown in the inequality of the idiochromosomes, which become progressively greater in the series (1) *E. servus*, (2) *tristigmus*, *fissilis*, (3) *ictericus*, (4) *variolarius*, the inequality in the last case being fully as great as *Lygæus*" (pp. 17). He demonstrates a marked difference in the relative size of the idiochromosomes of *E. variolarius* and *servus* in *a* and *c* of his Fig. 4.[1]

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[1] We have a large number of photographs of entire groups of chromosomes of *E. variolarius* and *E. servus* and we have also preparations from which we can photograph the chromosome groups of the F_1 and F_2 generations of this cross from both the testes and embryonic stages, but these results are reserved for a later publication, in which we shall give a complete account of our experiments in crossing *E. variolarius* with both *E. servus* and *E. ictericus*.

Hoping that such a marked difference in the chromosomes might be a constant feature, we anticipated being able to obtain some interesting comparative results by crossing the two species. We were disappointed, however, to find that in all the individuals we studied the inequality in the size of the idiochromosomes of *E. servus* is quite as marked as in *E. variolarius*, and that the chromosomes of these two species were therefore of no special value for this comparative study.

This seems to be another case pointing to inconstancy in the morphology of the chromosomes—as the individuals which Wilson studied showed a marked feature which was absent in the individuals studied by us. All such examples of inconstancy in vital processes throw additional light on the fact that investigators of the same material so frequently hold diametrically opposed views as to the fundamental significance of such expressions of vital activities, and that facts in support of these opposing views can be demonstrated in the same material.

This is almost amusingly illustrated by two recent papers on the cytology of certain Hemiptera, Gross ('12) and McClung and Pinney ('12). Gross differs from Wilson as to the facts on almost every point in which their hypothetical views clash. Of the cytological evidence for Wilson's theories, he says, "Geleitet von einigen Gesichtspunkten, die ich bei der Untersuchung von *Syromastes* und *Pyrrhocoris* gewonnen hatte, habe ich aus dem Studium der Literatur den Eindruck erhalten, das die scheinbar so sichere cytologische Basis von Wilson's Theorie hochst unzuverlässig ist. Ja, man kann mit gutem Grunde sagen, sie existiert eigentlich gar nicht."

He denounces Wilson's work on *Syromastes* as follows:

"Im einzelnen enthält Wilson's recht kursorische Darstellung zahlreiche, leicht nachzuweisende Lücken, Ungenauigkeiten und Irrtümer."

McClung ('12) condemns our work on *Anasa tristis* with quite as firm a hand, attributing to our methods of technique our failure to demonstrate the facts which he considers of most value. With the finality of a judge he hands down his decision: "It is my judgment that this method '*used alone*' is entirely inadequate for accurate results, and in this particular case is responsible for the discrepancy between these investigators and Paulmier, Wilson and Montgomery."[1]

A few facts will serve to convince any unprejudiced cytologist that our method of technique should not be condemned as "inadequate." From many of our preparations it is possible to demonstrate a large number of chromosome groups in which every chromosome is present and can be clearly photographed—in one case we have more than 150 photographs of such groups from the *same embryo*. A method that makes such results possible can hardly be condemned as "inadequate" for "accurate results"—it is a method, rather, that compels a recognition of enough variability in the same individual to make one very cautious in accepting premature hypotheses based on insufficient data.

In further condemnation of our methods McClung criticizes the value of photography as a means of demonstration. He says: "Foot and Strobell have employed photography alone as a means of presenting illustrations of their material, and it is assumed by them that if a thing can be photographed it must necessarily be a true picture of normal conditions. This I consider to be a decided fallacy. A photograph is an interpretation by the observer, just as is a drawing. The personal factor is no more absent from one method of illustration than it is from the other. Photographs may present with greater fidelity the details of structure in an object, but the choice of the object, and the nature of details are at the command of the photographer" (pp. 369).

We certainly do *not* assume that a photograph must of necessity represent only normal conditions, but we do believe that abnormalities are likely to be due to a pathological condition of the cells and that such a condition can quite unconsciously be obscured in a drawing, but not in a photograph. McClung's position is certainly unique when he claims that the personal factor is no more absent from a photograph than from a drawing and he bases this on the fact that an investigator may in either case *select* the object he wishes to photograph.

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[1] It is difficult to understand why McClung mentions and underscores "*used alone*" as on the same page he quotes from our paper in which we distinctly state that we use sections also for "comparative work."

It may be said that in every paper representing cytological research one or more hypotheses are on trial and it is certainly the privilege of the investigator to select for illustration such evidence in his preparations as support his own convictions in regard to the hypotheses under consideration.

Our skepticism of the sex-determination theory led us to study our preparations of *Anasa tristis* with a view to determining whether the phenomena involved in the theory were sufficiently constant to justify it. We found enough evidence to fully confirm our skepticism and we claim that we had the right to select that evidence from our preparations and give it, with as much emphasis as possible.

The facts are simple and clear. We demonstrated that in *Anasa tristis* the reverse of the facts demanded by the sex-determination theory were clearly present and could be illustrated by photographs. The interesting feature in these opposing observations is the fact that we do not deny the observations of our opponents, whereas their belief in the causal nature of the chromosomes compels them to set our observations aside or to resort to strained explanations in order to account for them.

It is the old story so familiar to cytologists—if a feature is where, hypothetically, it ought not to be, it is an artifact, and if it is not where it ought to be, it is due to faulty technique.

We believe that the photographs of our preparations prove conclusively that the accessory chromosome in *Anasa tristis* does not always fail to divide in the second spindle and the case may safely rest on a comparison of McClung's photographs of his preparation with ours.

We claim that the facts we have demonstrated in *Anasa tristis* are at least worthy to be placed in evidence against the chromosome theories that necessitate such facts being thrown aside as artifacts or as expressions of a pathological condition. We hold that they have at least the same value as normal variations in other organs of the cell and that they should be accepted as the same type of evidence we gave in '05 against the theories based on definite forms of the chromosomes. We demonstrated a degree of inconstancy in the form of the chromosomes of *Allolobophora fœtida* that should not exist if the hypotheses were valid.

It is interesting that such an experienced student of the cytology of the Hemiptera as Gross ('12) should also find that there are facts in *Anasa tristis* that are out of harmony with the theories. He writes:

"Später bin ich dann durch die Freundlichkeit der Herrn Dr. E. R. Downing noch in den Besitz einiger Präparate von *Anasa tristis* gelangt, deren Studium mir gleichfalls zeigte, das Wilson's Theorie mit den Tatsachen schwer vereinbar ist."

Nucleolus.—In the few Hemiptera we have studied we have interpreted the chromatin nucleolus of the first spermatocyte as the homologue of the nucleolus of other forms.

This is due to the fact that we have failed to find any other structure in the cell which we have felt justified in interpreting as a nucleolus. We were wrong in interpreting the chromatin nucleolus in *Anasa tristis* as independent of the accessory chromosome, an interpretation we were first led to doubt in our study of *E. variolarius* '09, and we stated in giving our results in this form that we would reserve the publication of the evidence until we could control it by a comparison with other forms. Buchner's work ('09) on the accessory chromosome enabled us to harmonize our conflicting evidence on this point. We wrote: "In this paper Buchner's observations on the accessory chromosome appear to throw some light on certain conflicting facts observed by us in *Euschistus variolarius*. Buchner supports Wassilieff in observing that only part of the substance of the chromatin nucleolus gives rise to the accessory chromosome. This would seem to indicate that the chromosomes in question are evolved from a nucleolar mass of chromatin, thus homologizing this structure with the cases in which it is claimed all the chromosomes are evolved from a large nucleolus, leaving a nucleolar residue after the chromosomes are formed. In *Euschistus* we find cases in which both the idiochromosomes and a chromatin nucleolus are present at the same time. Such facts, added to those cases in which the size relations of the chromatin nucleolus do not agree with those of the idiochromosomes, raise the question as to the identity of the two structures, though these facts would not conflict with homologizing the chromatin nucleolus with the nucleolus, which in some forms is said to give rise to all the chromosomes."

If these investigators are right in claiming that the chromatin nucleolus is in fact a chromosome which retains its individuality and continuity through the growth period, then it is quite necessary that another structure should be found in these cells which can be homologized with the nucleolus of other forms. If one is obviously not present, some plausible explanation of its absence must be given. If it is not figured in drawings, then the observer can be accused of superficial and careless observation; but if it is absent in photographs, then the old scapegoat—faulty technique—is held responsible.

This is McClung's explanation of the fact that a second nucleolus is conspicuously absent from all our photographs of the growth period of the spermatocytes of *Anasa tristis*. He says: "Owing to the technique employed by them the plasmosome is practically destroyed." It is certainly highly improbable that a method that has not destroyed the plasmosome in the other forms we have studied should destroy this structure in these Hemiptera.

It is interesting to notice how the plasmosome of *Anasa tristis* has developed and waxed strong since the earlier investigators described and figured it. It was described as a pale body and figured accordingly, but in Pinney's recent drawings it has developed into a strong dense body fully as chromatic as the chromatin nucleolus. Although Wilson himself describes it as a "*pale*" body, McClung's photographs from Wilson's preparations show that the structure which McClung interprets as a plasmosome is not a pale body, but is fully as chromatic as the chromatin nucleolus. Here, as in other points where our conclusions are condemned, we are willing to rest the case on a comparison of McClung's photographs with ours.

Our belief that the chromatin nucleolus of the Hemiptera is the homologue of those nucleoli of other forms that are said to give rise to all the chromosomes has been greatly strengthened by our study of the spermatogenesis of *Euschistus crassus*. In this form there are two chromatin nucleoli, one of which gives rise to the idiochromosomes and the second gives rise to a pair of autosomes—the so-called ordinary chromosomes.[1]

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[1] These results were sent to press last February.

All such deviations from a method of development that has been claimed to be sufficiently rigid to justify far-reaching generalizations are a warning against formulating general laws that do not take into account all the facts.

If our results demonstrated in *Anasa tristis* be shorn of all their significance except their admission as normal variations, they still stand as a protest against premature generalizing. We expressed this in an earlier paper (1911) in reference to our demonstration that the accessory chromosome in *Anasa tristis* does not always fail to divide in the second spindle. "If the significance holds which has been attributed to the failure of this chromosome to divide in one of the two maturation divisions, then those cases in which it divides twice in *Anasa* must be set aside as pathological, the spermatozoa resulting from these divisions having no functional activity. Only to those who have endowed the chromosomes with causal attributes can variations in their behavior cause embarrassment. If we find them subject to marked variations it is a characteristic they have in common with other structures in the cell—the nucleolus, the centrosome, the mitochondria, the polar rings and other structures which have been found to be so variable that interest in speculations as to their possible causal significance has steadily waned, and those who believe the chromosomes are equally variable may justly suspect that the hypotheses surrounding these structures may be destined to the same fate as the speculations so long surrounding other cell organs, notably the centrosome."

Nearly all cytologists agree that experimental breeding has been and may ever be the most trustworthy test of hypotheses which are based on the morphology of the cell and which claim to offer a mechanical explanation of heredity.

The chromosomes of the Hemiptera are largely responsible for the modern chromosome hypotheses, for certain stages in their growth and development which are assumed to justify these hypotheses can be clearly followed and demonstrated. Those Hemiptera therefore which show the chromosome phenomena necessary to a given hypothesis are especially fitted to test its value by experimental breeding.

It would seem that a cross between two species of these Hemiptera, one of which contains an exclusively male character which is absent in the other, should furnish some trustworthy evidence for or against the hypothesis that the factors for determining sex are located in a definite chromosome of the so-called male and female-producing spermatozoa.

We believe that our results from this experimental work are in harmony with our cytological evidence on this point and that these results support the skepticism of those investigators who believe that the evidence given by the chromosomes does not warrant the assumptions that have endowed these cell structures with causal attributes.

NEW YORK,
December, 1912.

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Description of Plates.

In all cases only the ventral surface of the bugs is shown. The specimens in photos 2 to 6 are preserved in glycerine. The genital segment of each bug has been pulled out and cotton inserted behind the segment to hold it in position to show the entire ventral surface. The bugs are magnified about 1½ diameters.

The photographs are reproduced by the half-tone method which, unfortunately, is not entirely satisfactory for showing the delicate shades of difference in the intensity of the spot.

Photo 1. *Euschistus variolarius* male, showing the ventral surface and the clearly defined black spot always present on the genital segment of the males of this species.

Euschistus servus male, showing ventral surface and the genital segment without any trace of the black spot characteristic of *E. variolarius* males.

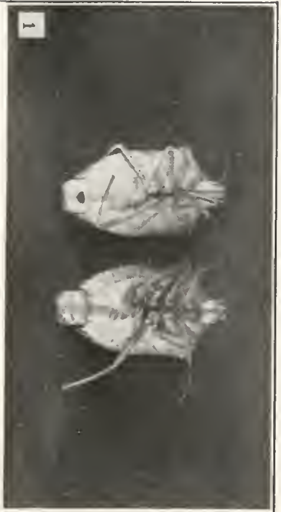
Photos 2 and 3. 8 of 46 males of the F_2 generation raised from a pair of F_1 hybrids, which were bred from an *E. variolarius* female raised in our laboratory, from a pair collected in Connecticut, and an *E. servus* male collected in North Carolina. Four of these F_2 individuals have no black spot on the genital segment, and four show the spot in a greater or less degree.

Photo 4. 7 of 54 males of the F_2 generation raised from a pair of F_1 hybrids, which were bred from an *E. variolarius* female collected in Connecticut and an *E. servus* male collected in North Carolina.

These F_2 individuals show the variations, from no spot on the genital segment, to one almost as pronounced as that of a pure *variolarius*.

Photo 5. *Euschistus variolarius* males, showing the typical black spot on the genital segment.

Photo 6. *Euschistus servus* males, showing the genital segments without the spot characteristic of *E. variolarius*.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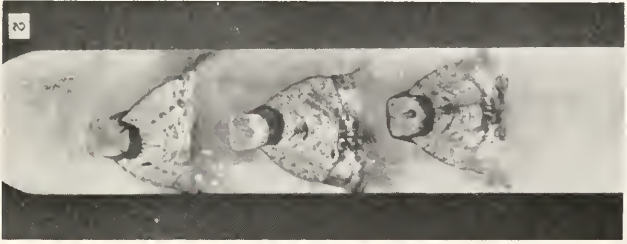
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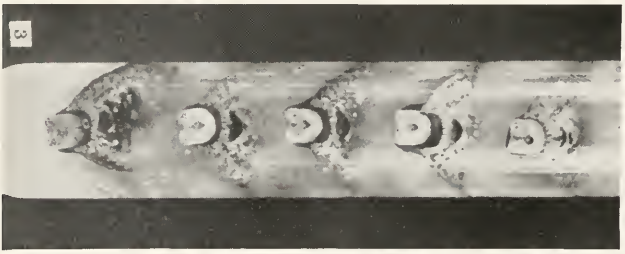
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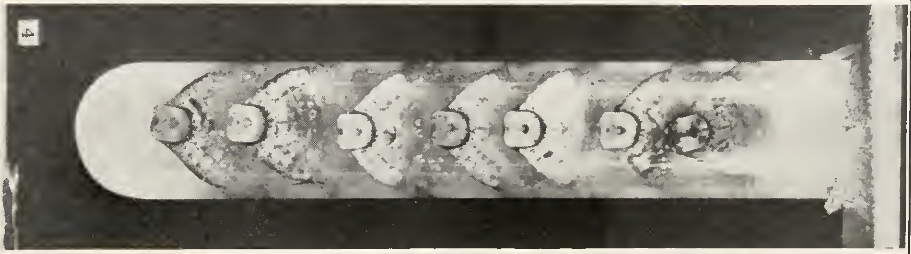
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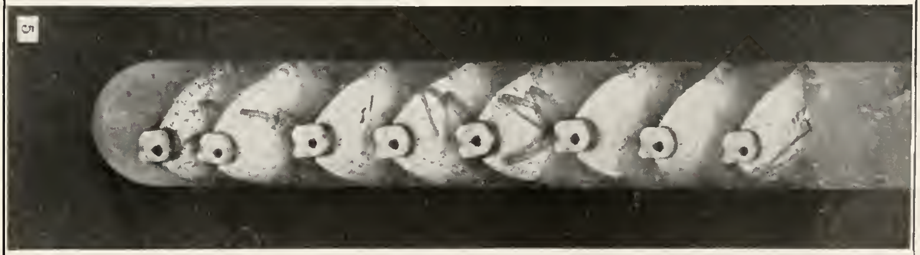
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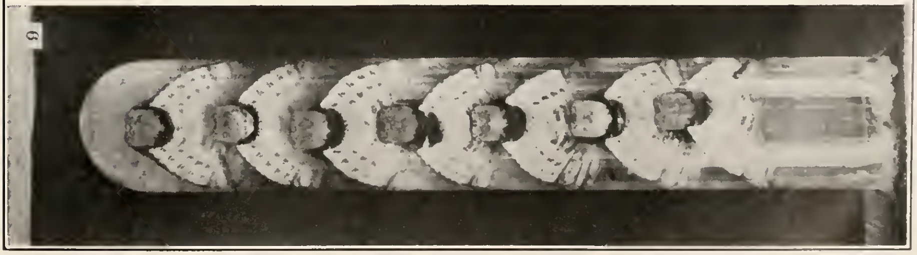
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Vol. XXIV. March, 1913. No. 4

BIOLOGICAL BULLETIN

DATA ON SEX DETERMINATION IN CATTLE.[1]

RAYMOND PEARL AND H. M. PARSHLEY.

INTRODUCTION.

In a paper from this Station published over twenty-two years ago Russell[2] presented certain statistics which he collected regarding the relation between the sex of a calf and the time in the period of heat (œstrus) at which the copulation occurred which gave rise to it. Data were collected in regard to (*a*) whether service occurred *early* in the "heat," or in the *middle* of the period of œstrus, or *late* in the "heat"; and (*b*) the sex of the resulting offspring. In the published results only the data for "early" and "late" coitus were presented. The figures given were as follows (*loc. cit.*, p. 209):

"82 cows served during the first part of heat produced 31 bull calves and 51 heifer calves.

"76 cows served during the last part of heat produced 42 bull calves and 34 heifer calves."

These figures were admittedly meager. After the paper referred to was published Dr. Russell continued the collection of statistics on this subject but published nothing further. Upon resigning from the Station staff he very kindly turned over to the senior author of the present paper all his original schedules. The complete collection of statistics has recently been subjected to analysis with the results set forth in this paper.

The original schedules contain spaces for the recording of the following information: (1) Breed and age of bull. (2) Breed of cow. (3) Age of cow. (4) Date of service. (5) Date of birth of calf. (6) Sex of calf. (7) Weight of calf. (8) "If you know positively, state whether the cow was served in the first, middle, or last part of heat." Space was provided on each blank for recording a year's breeding operations. These schedules were widely distributed among the leading stock breeders of the state, and the inquiry extended over a period of about four years. As in all such studies a portion of the blanks returned were defective and not usable, either through failure to fill in essential points or from mistakes. One of the commonest defects arose from the fact that the bull was allowed to run in the pasture with the cows, thus precluding any possibility of accurate knowledge of the period of œstrus at which coitus occurred. On the other hand the great majority of the returns were filled up in a very careful and painstaking manner, and furnish valuable data on a number of problems in the physiology of breeding which are much more in the foreground of biological interest at the present time than they were when the inquiry was instituted.

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[1] Papers from the Biological Laboratory of the Maine Agricultural Experiment Station, No. 42.

[2] Russell, F. L., "Breeding Statistics," Maine Agr. Expt. Stat., Ann. Rept. for 1891, pp. 208, 209.

All of the breeds of cattle at all common in the eastern United States are represented in the returns. The majority are either grade or cross-bred animals, but a fair proportion represent "pure bred," registered stock, particularly in the case of the dairy breeds.

In the present paper only a single problem will be discussed. In later communications it is hoped to take up certain other problems in the physiology of breeding for which data are available in these returns. The problem here considered may be stated as follows: Is there any relation and if so of what sort, between the time in period of œstrus at which coitus occurs and the sex of the resulting offspring? This is obviously a matter of much practical as well as theoretical importance. There is perhaps no one thing that would be of more vital importance to the stock-breeder than to be able to control, in some degree even, the sex of the animals which he breeds.

The visible manifestations and the duration of œstrus in the cow are rather variable matters. In the older literature, and in the minds of most practical breeders at the present time, no precise distinction is made between the two divisions of the œstrous cycle designated by Marshall[1] (p. 36) as the *Proœstrum* and *Œstrus* respectively. The combined period is called "heat" in some cases, whereas in others only a portion of the period of marked sexual activity is so designated. This fact, together with the natural variability of the phenomenon itself amongst different individuals, accounts for the rather widely varying statements found in the literature regarding the duration of "heat" in the cow. Leuckart[2] puts the duration for cow and mare at "about" 4 days. Thury[3] implies a rather shorter duration than this. Flemming[4] says regarding the matter: "The frequency and duration of the period of 'rutting' or 'heat' depends upon the age, species, and other circumstances; but it may be said to persist in the domestic animals from 1 to 15 days at the most. The shortest period is witnessed in the cow and sheep and the longest in the bitch. It is sometimes only present from 12 to 24 hours in some non-fecundated animals." Düsing[5] puts the time of active "heat" in cattle at 24–30 hours at the outside. This is the experience of practical breeders generally.

Under these circumstances it is not to be expected that the returns discussed in this paper are absolutely accurate as to the division of the œstrous period into early, middle, and later portions. No sharp delimiting boundaries between these divisions were, or could be, set in the schedules. The matter had to be left to the judgment of the breeder. We have not been able to discover any reason, however, for supposing the error introduced from the indefiniteness of the phenomenon itself to be biased in one direction or the other. There is unquestionably overlapping of the divisions of the œstrous period in the returns, but there is no reason to suppose that this is not as great proportionately in one direction as in the other. The relation between the returns and the true physiological division is probably substantially as follows:

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[1] Marshall, F. H. A., "The Physiology of Reproduction," London, 1910.

[2] Leuckart, R., "Zeugung." In Wagner's "Handwörterbuch der Physiologie," Bd. 4, pp. 707–1101, 1853.

[3] Thury, M., "Ueber das Gesetz der Erzeugung der Geschlechter bei den Pflanzen, den Thieren und dem Menschen," Leipzig, 1864.

[4] Flemming, Geo., "A Text-book of Veterinary Obstetrics," New York, 1879.

[5] Düsing, C., "Die Regulierung des Geschlechtsverhältnisses bei der Vermehrung der Menschen, Tiere und Pflanzen," *Jenaische Zeitschr.*, Bd. 17, pp. 593–940, 1884.

Time of Coitus as Recorded.	*Time of Coitus in Relation to Actual Stage in Œstrus of Individual.*
"Early."	Early + some in middle division.
"Middle."	Middle + some in early stage + an equal number in late stage.
"Late."	Late + some in middle division.

Finally there seems no reason to think that a psychological bias, conscious or unconscious, on the part of the breeders has influenced the returns. The opinions of breeders in regard to sex determination, so far as we have been able to get at them, are as follows: The majority of cattle breeders attach no significance to the time of service as a factor in influencing sex. Either they have never heard of any theory regarding the matter, or if they have are not disposed to attach any significance to it, or to modify or regulate their breeding practice in any way to conform to such a theory. A much smaller number of breeders have a decided opinion on the matter, which they usually believe to be supported in greater or less degree by their own experience. This latter class falls into two groups, about equal in numbers, and about equally dogmatic in their adherence to their particular views. One group believes that in order to secure a preponderance of females service must occur *early* in the "heat," whereas the other group maintains that to reach this end service must be *late* in the "heat." Thus one group offsets the other. Even from the men in these classes, however, the returns bear every evidence of being carefully and honestly made. An occasional somewhat rueful comment may be pencilled on the blank when the writer's theory failed to "work," but further than this preconceptions appear to have played no part.

The material would appear, from the biometrical standpoint, to be particularly favorable on the following accounts:

1. The sampling is strictly random. The different breeds are represented in substantially the proportions in which they

occurred in the State at the time of the inquiry. The statistics do not represent one or even a few large herds, but a large number of small herds scattered all over the State.

2. The original records were made by persons who knew nothing whatever of what use was to be made of them, or anything about what they "ought" to show in order to please or satisfy the person who collected them.

3. The numbers are sufficiently large to reduce the probable error of the sex ratios to reasonably small magnitudes. Of course, still more figures would be desirable, but the data as they stand are by far the most extensive yet collected on the point at issue. In the present state of knowledge regarding sex-determination it is desirable to submit to careful analysis any collection of statistics sufficiently extensive to be worthy the name.

Of course, any one considering the sex-determination problem from the statistical standpoint should be clear as to the nature of statistical evidence in general. Nothing can ever be finally "proved" by statistical evidence alone. All that any amount of statistical data can do is to state a *particular* set of facts, which are a sample, strictly localized in time and space, and restricted in magnitude, out of the whole universe of facts of like kind. If the counting has been correctly done the results from the sample are absolutely and unshakably *true, so far as concerns the sample itself.* Whether or not the sample fairly represents the relations in the whole universe to which it belongs, can never be *absolutely* determined, no matter how large the sample may be. All that can be ascertained is a more or less close approximation to the mathematical probability that the sample gives a just representation of the population or universe from which it came.

The most extensive statistics which have been published on the gross (*i. e.*, unanalyzed) sex ratio in cattle are those of Wilckens.[1] They embrace 4,900 calves and the sex distribution is 2,536 ♂ : 2,364 ♀, giving a sex ratio of 107.3 ♂ : 100 ♀.

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[1] Wilckens, M., "Untersuchung über das Geschlechtsverhältniss und die Ursachen der Geschlechtsbildung bei Hausthieren," *Landwirtsch. Jahrb.*, Bd. 15, pp. 611–654, 1886.

Data.

The sex distribution of 480 calves, in relation to the time of coitus in the œstrous period, is exhibited in Table I. Besides the absolute figure the sex-ratios calculated as number of ♂♂ per 100 ♀♀ and the percentages of ♂♂ and ♀♀ and their probable errors are given.

TABLE I.

SHOWING THE NUMBER OF CALVES OF EACH SEX, AND THE SEX RATIOS, FOLLOWING COITUS AT SPECIFIED TIMES IN THE ŒSTROUS PERIOD.

Time of Coitus.	Total Off-spring.	Sex of Offspring.		Percentage of		♂♂ to 100 ♀♀
		♂♂	♀♀	♂ Births.	♀ Births.	
Early in heat	248	123	125	49.60 ± 2.14	50.40	98.4
Middle of heat	125	67	58	53.60 ± 3.01	46.40	115.5
Late in heat	107	65	42	60.75 ± 3.19	39.25	154.8
Totals, all periods	480	255	225	53.13 ± 1.54	46.87	113.3

From this table the following points are to be noted:

1. The proportion of male births increases steadily with the later occurrence of coitus in the œstrous period.

2. Comparing the extremes there are more than 10 per cent. more males produced when coitus is late in heat than when it is early in the œstrous period.

3. Taking the three groups together the total figures give a sex-ratio intermediate between those exhibited in the extreme groups.

Now to consider the important question: Are the differences between the different groups shown in Table I., statistically significant, or are they such as probably arise from the inevitable "error" of random sampling? To get light on this point recourse must be had to the probable errors of the sex-proportions.

The probable errors given in the percentage column for the male births are calculated from the usual formula[1] for the probable error of a proportion or percentage, viz:

$$\text{P.E.} = .67449\sqrt{\frac{p\cdot q}{n}},$$

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[1] See G. Udny Yule's "An Introduction to the Theory of Statistics," London, 1911 (Griffin & Company), for a very clear discussion of this and other probable error formulæ.

where p denotes the proportion of "successes," q the proportion of "failures," and n the number in the sample.

Remembering that between two uncorrelated events the probable error of a difference is the square root of the sum of the squares of the probable errors of the quantities between which the difference is taken, we have the results set forth in Table II. From this table it is possible to form a first judgment as to the statistical significance of the raw data of Table I.

TABLE II.

SHOWING THE PROBABLE ERRORS OF THE DIFFERENCES BETWEEN CERTAIN OF THE SEX-RATIOS OF TABLE I.

Groups Compared.	Difference.	Probable Error of Difference.	Difference. / P.E. Diff.
Per cent. ♂ births "late in heat"—"early in heat"..	11.15	±3.84	2.90
Per cent. ♂ births "late in heat"—"middle of heat"..	7.15	±4.39	1.63
Per cent. ♂ births "middle of heat"—"early in heat"..	4.00	±3.69	1.08

From this table we note that in the case of the extreme groups the difference is 2.9 times its probable error. This means[1] that if the time of coitus in relation to œstrus were absolutely without influence on sex an excess of 11 per cent. of males such as is observed in the extreme case would only happen 5 times in every hundred that the matter was tested with statistical samples of the present size. Or, put in another way, the odds are 19 to 1 against the excess of ♂ births in "late in heat" matings being due to "chance" (*i. e.*, to errors of sampling) on the basis of probable errors here used. These are distinctly "long odds."

Can the probable errors calculated by the formula used above be relied upon to give correct results on data like the present? They assume normality of the error distributions. This assumption is practically justified in the present instance. The material may, however, be dealt with according to the method given by Pearson.[2] The present case may be regarded as falling in the category of estimating future expectation on the basis of experience furnished by a previous sampling. Pearson (*loc. cit.*) shows that only when a first sample is indefinitely larger than a second can we with entire accuracy take the probable error of the latter as $.67449\sqrt{mpq}$, when p and q are the chances of success and failure respectively in the former. He further shows that when a first sample n gives a percentage value of the character equal to $\bar{p}$, a second sample of m individuals may be expected to give

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[1] This statement regarding probabilities assumes that the errors of sampling for this material follow substantially the normal or Gaussian curve of error See below.

[2] Pearson, K., "On the Influence of Past Experience on Future Expectation," *Phil. Mag.*, March, 1907, pp. 365–378.

$$100\,p \pm 67.449\sqrt{\bar{p}q\left(\frac{1}{m}+\frac{1}{n}\right)} \text{ per cent.}$$

In the present instance we may take the cases of service early in heat as giving a "first sample" for the sex ratio in cattle, and then proceed to determine whether the groups served in the middle of heat and late in heat are to be regarded as random samples from the "early in heat" population.

We then have for the cases of service in middle of heat

$$\begin{aligned}\bar{p} &= .496,\\ q &= .504,\\ m &= 125,\\ n &= 248,\end{aligned}$$

whence the expectation for the percentage of ♂ births from cows served in the middle of heat, provided time or service had *no* influence on sex, any deviation being due to errors of sampling, is 49.6 ± 3.7. Or, as a result purely of random sampling a second sample from the "early in heat" population would be expected to show as often as not a ♂ sex percentage anywhere between the limits 45.9 and 53.3.

Actually the observed percentage of males for service in "middle of heat" was 53.6, practically the same as the upper limit given.

For cases of service in last of heat we have $m = 107$, and the other values as before. Whence it is deduced, by the same reasoning as before, that the expectation for the percentage of male births from cows served *late* in the period of heat, provided time of service had *no* influence on sex, any deviation being due to errors of random sampling, would be 49.6 ± 3.9.

This means that a random sample of the size of this "late in heat" groups, from a population having the sex ratio exhibited by the "early in heat" sample, would be expected as often as not to give a male sex percentage within the limits 45.7 and 53.5.

The observed percentage of male births in the "late in heat" lot was 60.75. This value is well outside the limits, and the probability against its being a result of random sampling is considerable. It is not, however, so great as to amount to certainty. The standard deviation of the error distribution for random samples of the size of this "late in heat" lot is approximately 6.2. Taking the rough rule that for practical purposes the half range as either side of the mean is 3 σ, it would give an upper limit to the curve of 67.9. The observed 60.75, while getting well out towards the range end, still lacks something of actually reaching that point, to say nothing of going beyond.

In general, the data presented appear to warrant the following conclusions: *So far as the present statistics are concerned there is an increasing proportion of male births as the time of service approaches the termination of the œstrous period.* This increase amounts to approximately 10 per cent. in the extreme case, and to not quite half this in the intermediate case. Statistically considered the difference in the extreme case is *probably* significant (*i. e.*, not due to errors of random sampling). The probability that the observed increase in the proportions of male births is a real and biologically significant phenomenon, while not enormously great, is nevertheless of an order which one would not hesitate to act upon in the practical affairs of life.

So much for the bare facts. We have now to consider the possibilities as to the cause of the observed relation. *Apparently* the increase in male births is associated with time of service, but before accepting such a conclusion it is important to determine whether some other variable factor may not be influencing the results. The first possibility which suggests itself is that there may be a difference in age distribution of the animals in the three lots and that in some manner this has affected the result. In the ideal experiment of this sort of course the parents used should be of the same average age in the different groups.

The age distribution of the animals involved in these statistical returns has been examined with the results shown in Tables III. and IV. Table III. gives the actual frequency distribution, and Table IV. the constants calculated therefrom. It should be said that in these distributions the parents are weighted with their fertility. That is, each individual appears once for each offspring. This seems the fairest manner in which to deal with the problem, for comparisons such as are here indicated.

TABLE III.

SHOWING THE AGE DISTRIBUTION OF THE ANIMALS IN THE SEVERAL GROUPS.

Age in Years.	Service Early in Heat.		Service Middle of Heat.		Service Late in Heat.	
	No. of ♂♂.	No. of ♀♀.	No. of ♂♂.	No. of ♀♀.	No. of ♂♂.	No. of ♀♀.
1– 1.9	71	7	44	4	25	2
2– 2.9	68	26	26	15	49	12
3– 3.9	39	31	16	11	13	15
4– 4.9	8	23	4	13	2	13
5– 5.9	17	16	5	12	1	8
6– 6.9	2	30	1	10	2	12
7– 7.9	—	27	—	7	—	12
8– 8.9	—	13	—	10	—	11
9– 9.9	—	8	—	6	—	8
10–10.9	—	16	—	6	—	5
11–11.9	—	7	—	2	—	2
12–12.9	—	4	—	6	—	1
13–13.9	—	2	—	—	—	1
14–14.9	—	0	—	—	—	—
15–15.9	—	1	—	—	—	—
Totals[1]	205	211	96	102	92	102

From these distributions the constants of Table IV. have been calculated by the ordinary biometric methods.

TABLE IV.

SHOWING THE VARIATION CONSTANTS FOR THE AGE OF BREEDING CATTLE.

Group and Sex.	Mean, Years.	Standard Deviation, Years.	Coefficient of Variation, Per Cent.
Service early in heat—♂♂	2.71 ±.06	1.21 ±.04	44.7
Service middle of heat—♂♂	2.49 ±.08	1.16 ±.06	46.6
Service late in heat—♂♂	2.53 ±.07	.93 ±.05	36.6
Service early in heat—♀♀	6.16 ±.14	2.95 ±.10	47.9
Service middle of heat—♀♀	6.15 ±.20	3.04 ±.14	49.5
Service late in heat—♀♀	6.15 ±.18	2.73 ±.13	44.4

[1] The differences between these and the offspring totals are due in the main to failures in individual cases to record the age of an animal in the schedules. In small part they are due to multiple gestation.

Obviously there are no differences in age distribution of sufficient magnitude to cause the observed differences in sex-ratios among the three groups. The males used in the three groups are of very nearly the same mean age. The same is true to an even more marked degree of the females of the three groups. It would be difficult to collect a more homogeneous lot of statistics than the present one, so far as concerns age distribution of the individuals in the several samples.

We have not been able to discover any other factor which could account for the observed differences in the sex-ratios described above. By the most careful tests which we have been able to apply the material appears to be statistically homogeneous except in respect to the time of service.

Some of the individual records are of interest. The following schedule from Mr. C. E. Clifford may be cited in particular. He was a breeder of registered Jersey cattle and carefully followed a regular system of service in relation to time of heat. This method in his own words was as follows: "Cover cow twice *immediately* upon discovery of heat and keep her separate from all [other cattle] until heat is entirely passed." The sex distribution for 14 calves born in the year for which the return was made was 2 ♂♂ and 12 ♀♀.

DISCUSSION.

It will have been noted that the results set forth above support, in essential features, the theory of Thury (*loc. cit.*) as to sex determination in cattle. Thury's original paper was published in French under the title "Memoir sur la loi de production des sexes chez les plantes, les animaux et l'homme." It immediately attracted the attention of biologists and was critically reviewed and discussed in the *Zeitschrift für wissenschaftliche Zoologie* (Bd. XIII., Heft 4) by Pagenstecher. He later translated Thury's paper into German, added appendices, etc., and it is this German translation which has chiefly been cited by later workers. The essential features of Thury's theory, so far as concerns mammals normally bearing a single young at birth, were set forth in the following words (Thury, *loc. cit.*, p. 16).

"Das Geschlecht hängt ab vom Grade der Reifung des Eies im Augenblicke, wo es von der Befruchtung getroffen wird.

"Das Ei, welches, wenn es befruchtet wird, noch nicht einen gewissen Grad der Reifung erreicht hat, giebt ein Weibchen; ist dieser Grad der Reifung überschritten, so giebt das Ei, wenn es befruchtet wird, ein Männchen.

"Wenn zur Zeit der Brunst, ein einziges Ei, vom Eierstock abgelöst, langsam durch den Geschlechtsapparat herabsteigt (Thiere, welche ein Junges gebären), so genügt, es dass die Befruchtung am Anfange der Brunst statthabe, um Weibchen zu zeugen, und am Ende, um Männchen zu zeugen, indem die Umwandlung (vire) des Zustandes des Eies normal während der Dauer seines Durchganges durch den Geschlechtskanal stattfindet."

Curiously enough this theory, while exciting much interest at the time of its appearance, has never been given any adequate experimental-statistical test. The only experimental evidence which Thury himself offered in its favor was that obtained by a friend Cornaz[1] which was so brilliantly confirmatory as to arouse suspicion in the minds of all subsequent workers, with the result that it has never been regarded as of any particular worth. At the best it was ridiculously inadequate statistically. Cornaz states that by following Thury's directions as to time of service he obtained 22 female calves in unbroken succession. In 7 cases he desired and obtained male calves, the service in these cases being late in the heat.

Düsing (*loc. cit.*) discussed Thury's theory at great length, and pointed out in the following words one of its obviously great weaknesses (p. 16): "Indessen ist diese eigentliche Thurysche Theorie in sofern nicht richtig, als immer nur ein gewisser Überschuss des einen oder andern Geschlechtes erwartet werden kann." Thury postulated *complete* determination.

Later writers have dismissed Thury's theory with rather scant consideration. Thus F. H. A. Marshall (*loc. cit.*, p. 630) says: "Thury claimed that he could regulate the sexes in cattle by allowing coitus only at the beginning or at the end of œstrous periods, . . . but other investigators have failed to establish Thury's conclusions." Morgan[2] (p. 393) says: "Thury based

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[1] Published as a part of Thury's paper (*loc. cit.*), pp. 17 and 18.

[2] Morgan, T. H., "Experimental Zoölogy," New York, 1907.

based his conclusions on 29 experiments with cows, in which union took place at the beginning or at the end of the period of heat. Others have failed to confirm his conclusions, and contradictory results have been obtained with rabbits, and hens." Neither Marshall nor Morgan give any detailed references to the literature.

F. R. Marshall[1] evidently has Thury's theory in mind in the following statement but has apparently received the tradition in slightly garbled form: "The commonest idea about sex determination is that females bred at the beginning of the period of heat produce male offspring. Other notions are based on the same supposed principle, namely, that an ovum fertilized while immature produces a male; maturity is supposed to be in proportion to the age of the ovum and the nutritive condition of the dam." He then proceeds (on grounds it must be said which are strictly *a priori*) to demolish all such notions.

So far as we have been able to discover the following are the only experimental tests which have ever been accorded Thury's theory as regards cattle breeding, which at all adequately fulfill the conditions necessary for a critical test. Supposed tests of the theory carried out with plants, multiparous mammals, birds, etc., will not be discussed here, because it seems to us either (*a*) obvious *a priori* that the theory could not possibly apply, in the nature of the case, to some of the organisms and forms of reproduction which Thury and subsequent workers attempted to include in its purview, or (*b*) that the so-called tests of the theory were in many instances absolutely uncritical and could not have been expected under the most favorable circumstances to have yielded any really significant results one way or the other. The tests with cattle, which if carefully carried out must yield critical evidence, are, as has been said, meager enough in number and magnitude. The first were made shortly after the publication of the theory at the agricultural academies at Proskau and Eldena.[2] The results were as follows:

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[1] Marshall, F. R., "Breeding Farm Animals," Chicago, 1911.

[2] *Annalen der Landwirtschaft*, Jahrg. 23, Bd. 46, p. 271, 1865. For the facts regarding these and the following tests we are indebted to Düsing's (*loc. cit.*) memoir.

Time of Service.	Sex of Young. ♂	♀
Early in heat	8	10
Late in heat	4	1

From these figures it was concluded that the theory in the bald form proposed by Thury was untenable.

The matter was again tested in Waldau and Eldena[1] with the following results:

Time of Service.	Sex of Young. ♂	♀
Early in heat	3	10
Late in heat	1	1

The last experimental test of the matter of which we have been able to find any report (except that of Russell, *loc. cit.*) is that of Touchon[2] who obtained the following results.

Time of Service.	Sex of Young. ♂	♀
Early in heat	0	11

These figures are very far from extensive. In view of the great practical importance of the matter, however, one would have thought that they would have acted as a stimulus to more adequate researches.

It is worth while to put together all the data regarding the relation of time of service to sex in cattle. We have the following figures:

Time of Service.	Sex of Young.		♂♂ : 100 ♀♀.
	♂	♀	
Early in heat	134	178	75.3
Middle of heat	67	58	115.5
Late in heat	77	44	175.0
Totals	278	280	

These figures are suggestive. While they certainly demonstrate that Thury's theory in its original form is untenable, on the other hand, they strongly indicate, though they admittedly do not absolutely *prove*, that the sex ratio in cattle may be modified by controlling the time of service in relation to the œstrous period. We would call attention to the fact that the totals give approximately a 1 : 1 ratio, as on *a priori* grounds they should.

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[1] *Annalen ner Landwirtschaft*, Wachenblatt, 1866, p. 461.

[2] *Agronomische Zeitung*, 1865, p. 519.

Granting the fact that the sex ratio in cattle does change as the time of service changes, what is the biological basis of the phenomenon? Thury's idea was that the sex of the offspring was determined by the relative staleness of the ova. In making this hypothesis it may be assumed either that ovulation occurs early in œstrus entirely, or as a more or less regular process throughout the œstrous period. Unfortunately no exact and thorough studies have been made on the physiology of reproduction in the cow. As has been pointed out by Marshall (*loc. cit.*, p. 316) the fact that artificial insemination has been successfully practiced with cows would indicate that ovulation is independent of coition. In a later paragraph that author states that: "There can be little doubt that in the great majority of mammals ovulation, as a general rule, occurs regularly during œstrus." This would certainly seem to be the case in the cow, since our statistics show that pregnancy may follow coitus at any time during the œstrous period. It has lately been contended by Jentsch,[1] on rather meager evidence, that cows even when served out of the œstrous period will in some cases become pregnant. The duration of life of the unfertilized egg in the uterus is not known for the cow. It has recently been shown by Lewis[2] to be distinctly brief in the case of the sow. He states as the result of numerous observations and experiments that the ovum "does not retain its vitality for more than a few hours after being liberated from the Graafian follicle." But if in the cow ovulation begins at or very near the beginning of œstrus, as seems certain from the returns in some of our schedules, where pregnancy followed a coitus within one or two hours of the beginning of œstrus, then it can fairly be considered as probable that the average age of fertilized ova following coitus late in heat will be somewhat greater than that of the ova fertilized early in heat. Nothing more than an average difference can be claimed (*i. e.*, not every individual ovum fertilized late in heat will be older than every individual fertilized early) and it is not possible to make any accurate estimate of what the difference amounts to. However, until some more definitely related factor is found it may be tentatively concluded, purely as a working hypothesis, that the observed changes in the sex ratios are correlated with changes in the relative freshness (or staleness) of the ova at the time of fertilization.

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[1] Jentsch, A., "Ueber Befruchtung ausserhalb der Brunstzeit beim Rind," *Jahrb. wiss. prakt. Tierzucht.*, Bd. 6, pp. 441–444, 1911.

[2] Lewis, L. L., "The Vitality of Reproductive Cells," Oklahoma Agr. Expt. Sta. Bulletin 96, pp. 1–47, 1911.

The hypothesis that the metabolic condition of the ovum when fertilized may influence the sex of the offspring finds support in the recent work from Richard Hertwig's laboratory. Hertwig[1] in a series of studies on frogs found that over-ripeness of the eggs was associated with a preponderance of males, extending in favorable cases to the production of 100 per cent. males. These results were criticized on several grounds. The experiments, however, have been very carefully repeated and extended by a student of Hertwig's, Kuschakewitsch,[2] who obtained similar results. The experiments were criticized by Morgan first on the ground of differential mortality, and second on the ground of differential fertilization, but Kuschakewitsch was able to show that the results were not open to criticism on these grounds. Whatever the explanation the *facts* brought out by Hertwig and Kuschakewitsch must certainly be accepted. The procedure which they followed led to a great preponderance of males. Miss King[3] has also shown that it is possible to modify significantly the sex-ratio by changing the metabolic condition of the eggs.

In the last few years a considerable body of evidence has accumulated showing that sex determination is primarily a matter of inheritance. It is not necessary to review the voluminous literature here. Essentially two points have been demonstrated. On the one hand the cytologists have shown for many forms that certain chromosomes are definitely associated with sex differentiation. On the other hand, the geneticists have shown that in a considerable number of forms the phenomenon of sex-linked inheritance is exhibited, a phenomenon which has as yet received no more cogent explanation than the obvious one that sex differentiation depends upon germinal factors, and that these behave in inheritance in accordance with Mendelian principles.

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[1] Hertwig, R., "Ueber das Problem des sexuellen Differenzierung," *Verhandl. deutsch. Zool. Ges.*, 1905; "Weitere Untersuchungen über das Sexualitätsproblem," *ibid.*, 1906; "Weitere Untersuchungen," etc., *ibid.*, 1907.

[2] Kuschakewitsch, S., "Die Entwicklungsgeschichte der Keimdrüsen von *Rana esculenta*. Ein Beitrag zum Sexualitätsproblem," *Festschrift f. Hertwig.*, Bd. 2, 1910.

[3] King, Helen Dean, "Studies on Sex Determination in Amphibians, IV. The Effects of External Factors, Acting Before or During the Time of Fertilization, on the Sex Ratio of *Bufo lentiginosus*," BIOL. BULLETIN, Vol. XX., pp. 205–234, 1911. See also others paper in the same series.

No one could rate higher the evidence that sex is determined primarily by inheritance than does the writer. But it is idle to deny that there is also a large and increasing body of critical evidence indicating that, in one way or another, sex ratios may be modified experimentally, and to some degree indeed controlled. Any adequate hypothesis of sex-determination must account both for the facts indicating innate pre-determination in the germ cells before fertilization, and also those indicating the modifying influence of external factors. Because one of these sets of facts is true does not mean that the other set is necessarily false, and should be forthwith annihilated (if possible) by destructive criticism. Such definite facts cannot be mutually exclusive. It would seem that a logical view of the case is that while sex is, in many cases at least, *primarily* determined by innate hereditary causes, nevertheless external factors, acting at the appropriate time, may in some instances modify the effect of the innate factors. This is essentially the same conclusion as is reached by Schleip[1] in his recent extensive and critical review of the literature in this field.

It is not difficult to conceive how such results could be brought about physiologically. The evidence indicates that particular chromosomes are concerned with the hereditary determination of sex. Furthermore a broad survey of the experimental work in the modification of sex-ratios by external conditions indicates that the most effective agencies in bringing about such modifications are those which directly affect the metabolic condition of the germ cells (*e. g.*, staleness, extraction of water, etc.). Why may not the sex chromosomes be affected by these agencies along with the rest of the germ cell to a sufficient degree to modify their specific effect? As has recently been pointed out:[1] "There are two ways of looking at the relationship between sex chromosomes and primary and secondary sexual characters on the assumption already made that the differential factor is essentially quantitative in nature. On the one hand it may be assumed, as it has been in most discussions of the subject, that the X-chromatin acts in a *positive* way, if at all, in the determination of sex. On this view two 'doses' of X-chromatin, in some manner not fully understood, positively determine the development of female characteristics. It has been argued that since the female is primarily anabolic in tendency, as pointed out many years ago by Geddes and Thomson, the determination of the female sex by a 'plus' condition in respect of chromatin may be explained on the assumption that X-chromatin is a sort of 'tropho-chromatin' in which reside the energy potentialities of the organism.

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[1] Schleip, W., "Geschlechtsbestimmende Ursachen im Tierreich," *Ergeb. u. Fortschr. Zool.*, Bd. III., pp. 165–328, 1912.

"There are two difficulties which confront this interpretation. The first is that the female characters cannot be regarded as an extension or intensification of those of the male. Rather the contrary is true. The male almost universally represents a higher degree of specialization and differentiation in development than the female. Another difficulty is found in the phenomenon of hermaphroditism.

"I would suggest that both of these difficulties may be overcome and all of the facts be better interpreted by assuming that X-chromatin is not a positive cause of sex differentiation but rather is an *inhibitor* of the development of male sex characters. It is a well-known fact that in the vertebrates, where the embryology of the genital organs have been most carefully studied, the male condition represents a more extended and advanced degree or stage of development than the female. The system is in every part homologous in male and female, but the latter appears objectively to have been arrested in a development which, without such arrest, would have led to the same result as is seen in the male. The same consideration applies to the male and female germ cells. The ovum is much less widely differentiated from the primordial indifferent germ cells than is the spermatozoön. Now if it be assumed that two 'doses' of this specific X-chromatin serve to inhibit completely the development of 'maleness,' while one 'dose' is insufficient to do this, but allows the male characters to develop, we shall have, it seems to me, a more satisfactory interpretation than is gained by looking at the matter in the reverse way.

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[1] Pearl, R., in as yet unpublished lectures on the "Biology of Sex," delivered before the Graduate School of Agriculture, Lansing, Michigan, July 1–6, 1912.

"This viewpoint would explain why it is that castrated males do not, save possibly in exceptional circumstances, take on female characters. On the other hand, according to Goodale's observations, castrated females do take on male characters. Further an explanation is found for the numerous cases in which a female in old age or after disease takes on male characters.

"On this view hermaphroditism becomes analogous to the phenomenon of the retention of larval characters in development it being assumed that there is one 'dose' or less of X-chromatin in such cases."

On either of these hypotheses as to the sex-determining action of the X-chromosomes it is not difficult to conceive how any change in the general metabolic condition of the germ cells might modify the sex ratio. The X-chromatin would presumably be affected along with the rest of the cell and the relative potency of its sex-determining factors changed.

In conclusion we wish our position in regard to the results set forth in this paper to be clearly understood. It is not contended or supposed by the writers that the time of service in relation to the period of heat *absolutely controls* the sex of the subsequent offspring. It is believed, however, that the facts set forth show, with a considerable degree of probability, that the sex ratio in cattle can be to some extent *modified* by controlling the time of service. But the amount of such observed modification is not so great that the matter can be tested with a few individuals. There is every reason to believe that any effect would only appear in fairly comprehensive statistics. The matter is one of much practical consequence to the stock breeder. Because this is so we would caution the reader against misinterpreting the results of this paper. A trial on a half dozen individuals will not in any sense whatever adequately test the accuracy of the results set forth in this paper. Nor will a breeding experiment with any other animal than the cow. If any person chooses to generalize from the data of this paper that in animals in general, or indeed in any other animal than the cow, time of service and sex of offspring are causally related, the responsibility for such generalization must be *entirely* his, not ours. We have studied statistics for cows in regard to this problem, and as yet no others, and have tried to make clear just what these data show. Beyond this solid ground of fact we do not care to venture, particularly so far as concerns the practical application of these results by the breeder.

Time of service is very evidently no absolute determining factor for sex in cattle. On the other hand the probability that the sex ratio can be changed by careful attention to this matter of time of service is sufficiently great, in our judgment, to warrant any man in modifying his breeding practice in accordance with it, particularly since in so doing he will be incurring no added risk of any kind. In the every-day affairs of life in regard to business, investment of funds, and the like, practical men every day undertake courses of action on the basis of probabilities much smaller than that in favor of getting an increased number of males if cows are served late in the heat. The practical cattle breeder in most cases would like, if he could get it, an excess of female calves. All the evidence at hand warrants the belief that by taking care that cows are served *as soon as possible* after the onset of heat there will be some reduction in the proportion of male calves born. In short, the facts set forth in this paper warrant the breeder in paying attention to the time of service in his cattle breeding operations, but he must not suppose that by so doing he can absolutely control the sex of the offspring, or even approach measurably close to absolute control. He can at best merely modify, over a period of years, the sex ratio in greater or less degree, in the direction which he desires.

SUMMARY.

In this paper statistics collected some years ago at the Maine Agricultural Experiment Station in regard to the relation between

time of service in the œstrous period and the sex of the subsequent offspring in domestic cattle are subjected to biometric analysis. These statistics are much more extensive than any which have hitherto been collected for the study of this problem in cattle. It is shown:

1. That as the time of coitus approaches the end of the œstrous period there is a progressive increase in the proportion of male young born.

2. That in the extreme case this increase in the proportion of male births is probably statistically significant and not to be attributed to errors of random sampling.

3. That these modifications of the sex ratio cannot be attributed to age differences or to any other factor yet suggested.

A possible explanation of the results and their practical bearings are discussed.

VARIATIONS IN THE SIZE OF CHROMOSOMES.

FRANK A. HARTMAN.

Outline of Contents.

I. Introductory 226
II. Material 226
III. Observations 227
  1. Comparison of the size of chromosomes 227
    (*a*) Chromosomes of nymphs compared with those of adults 227
    (*b*) Comparison of chromosomes of different cells of the same individual 228
  2. Causes of chromosome size variations 228
    (*a*) Unequal growth 228
    (*b*) Unequal division 229
IV. Discussion 230
  1. Chromosome variations and their causes 230
  2. The rôle of chromosome variations in heredity. Theoretical 230
V. Conclusion.—The facts and the theory which they suggest 231

I. Introductory.

From the time that the importance of germ cell chromosomes was first realized, the mass of investigation on this subject has dealt with the development of the chromosomes. Practically nothing on a comparison of chromosomes of the same species has been published. Therefore these results are given that they may throw some light on the behavior of chromosomes.

The observations in this paper, although somewhat limited, suggest interesting theoretical considerations.

Further investigations are being made by the author to test these ideas.

II. Material.

All of the material is of the species *Schistocerca americana*, with the exception of one specimen which is *S. alutacea*. A brief description of the material follows.

Young Nymphs.—Out of a number of nymphs, collected at the same time and place near the Kansas University campus, nine were taken at random. These were probably of the same age, for they were of approximately equal size, ranging from 12 to 14 mm. in length.

Older Nymphs.—Two of them, one 26 mm. long, wing stubs 6 mm.; the other 33 mm. long, wing stubs 9 mm. Both were collected by W. R. B. Robertson in Dickinson Co., Kansas.

Adults.—Two were furnished by Mr. Robertson from the locality mentioned above. Three were furnished by the Kansas University Zoölogy Department, two of them being *S. americana* and the other *S. alutacea.* The average measurement of dried adult specimens was 44 mm. exclusive of wings.

All material was fixed with Flemming and stained with Heidenhain's iron hæmatoxylin.

III. Observations.

1. *Comparison of Chromosomes.*

(*a*) *Chromosomes of Nymphs Compared with Those of Adults.*—The greater size of the chromosomes of adults as compared with those of nymphs is most easily seen in the first spermatocyte stages, as there the chromosomes are the largest of any period in their history. Great contrast in the size of chromosomes appears in both metaphase and anaphase, provided the nymphs are young enough. The nymphs, showing the greatest contrast were less than one third grown.

In first spermatocyte metaphase the chromosomes of young nymphs occur more in the spherical or short rod form than as rings or twisted rods which in adults are common. Large chromosomes are usually doubled or coiled and as there are not so many large ones in the nymph cells naturally the number of these complicated forms is smaller.

A large number of drawings were made as carefully as possible with a camera lucida. A few of these were selected in illustrating this paper. They are all typical of the material studied.

The twelve chromosomes within a cell were numbered according to their size. In almost every case the corresponding chromosomes of adult cells were larger than those of young nymph cells. The exceptions to this, although few, occurred among the chromosomes of smaller size (see Plate I., Figs. 1 and 2, 3 and 4, 5 and 6, 15 and 16). In some instances there was great contrast in size (see Plate II., Figs. 25 to 32).

In first spermatocyte anaphase the same comparison holds good with the exception of the chromosome form. The V- or U-shaped chromosomes of young nymphs are shorter and thicker than those of adults (see Figs. 33 to 47, Plate II.).

In the second spermatocyte metaphase there is a repetition of the size differences discussed in connection with the first spermatocyte (see Figs. 48 to 55, Plate III.). In connection with this it should be stated that almost invariably the cells of the young nymphs were smaller than those of adults.

(*b*) *Comparison of Chromosomes of Different Cells of the Same Individual.*—One of the most interesting conditions appeared in different cells of the same individual. Chromosomes which correspond as the number ones of the different cells show in many cases variations in size (compare corresponding chromosomes of the groups in: Figs. 1, 3, 6, 8, 9, 11, 14, 16; Figs. 2, 4, 5, 7, 10; Figs. 12, 13; Figs. 17, 19, 22, 24, 25, 27, 30, 32; Figs. 18, 23; Figs. 20, 21; Figs. 26, 28, 29, 31; Figs. 56, 57; Figs. 58, 59; Figs. 60, 61, 62, 63). In some instances this difference in size is very striking (see ones in Figs. 5 and 7; nines in Figs. 12 and 13; ones, twos, and threes in Figs. 22 and 24; and many others which are evident by a comparison of the figures).

A great number of cells were studied to find whether this size variation was exceptional. The variation was not unusual, but very common.

The first spermatocyte anaphase presented like differences in size (see Figs. 33, 35, 37, 39, 41, 43, 47; Figs. 34, 38, 40, 42).

Chromosmes in the second spermatocyte metaphase show similar variations (Figs. 48, 50, 55, 49, 51, 54).

The second spermatocyte anaphase chromosomes are just as variable (Figs. 64, 65, 66, 67).

There is no doubt that corresponding chromosomes of the same individual show a tendency to vary in size. This tendency becomes more marked in certain cells.

2. *Causes of Chromosome Size Variation.*

(*a*) *Unequal Growth.*—During early prophase of the first spermatocyte it is well known that the chromosomes appear as groups of granules, which may be more or less clearly distinguished from each other.

One slide which had most of the stain removed contained a number of cells of the early prophase with only one group of granules showing distinctly. The other granules were either very faint or else did not show at all. The granules of this group varied in size and number and in many cases were in the process of division (Figs. 69, 70, and 75 to 80). When the cells grew older, as could be determined by the increase in size of the cell and the appearance of the spiremes of the other chromosomes, the granules of this group began to come closer together and finally fuse (see Figs. 71 to 74 and 82).

The body produced by this fusion is unquestionably a chromosome because it is made up of granules which have the power of division. It is not the accessory chromosome for the accessory is present in practically every cell containing it.

The chromosome appearing in this series of observations seems to vary in size as commonly found among corresponding chromosomes of the same individuals (described above) (compare Figs. 73 and 74).

The number of granules in each group where no fusion had commenced was as follows: 7, 9, 9, 14, 9, 10, 8, 9, 11, 10—between 9 and 10 seemed to be the average. The fact that some granules had not divided could account to a certain extent for the differences in number, but not in every case, as Fig. 70, in which but one granule is undivided would give but 10 granules if this were divided. An examination of the other groups will show like discrepancies. Therefore the number of granules varies independently of their condition of division.

The variation in the size of the granules in cases where fusion had not occurred, was considerable. Even where all of the granules had divided they varied in size (see Fig. 75, as well as many of the other groups figured). This is true whether we compare granules of the same cell or of different cells (see Figs. following 68 and 75).

(*b*) *Unequal Division.*—In some of the lateral views of the first spermatocyte a few small chromosomes were found dividing unequally (Figs. 85 to 91). One cell contained two chromosomes dividing unequally (Fig. 88). Small chromosomes were found dividing unequally in the second spermatocyte (Figs. 83 and 84).

There is no question then that unequal division does sometimes occur in chromosomes.

IV. DISCUSSION.

1. *Chromosome Variations and Their Causes.*

The difference in size of chromosomes of nymphs as compared to adults depends upon the age of the nymphs. It also bears a direct relation to the size of the cells, the smaller cells of nymphs containing the smaller chromosomes.

Cells of young nymphs are dividing more rapidly than are those of adults, for that reason they probably have not the chance to grow so large. In addition to this the body cells in young animals undoubtedly develop at the expense of the germ cells because it is more important that the body organs become established before the germ cells are brought into activity. Then when the body has almost reached its growth the germ cells get their share of nutrition and become larger as do the chromosomes.

A study of the growth stages of a corresponding chromosome, in the same individual shown in Figs. 68 to 82, has demonstrated that the number and size of the granules differ. Either is sufficient cause for a variation in size of corresponding chromosomes.

Unequal division likewise produces unequal chromosomes.

These two facts lead us to expect unequal corresponding chromosomes in the same animal. A careful study proves that this is of common occurrence, regardless of the species concerned.

2. *The Rôle of Chromosome Variations in Heredity.*

Theoretical.—It has been believed for a long time that the chromosomes of the germ cells play the most important part in heredity. Their individuality seems to be established as the result of the work of many investigators. The picture which we now have of each individual germ cell chromosome is a body which is able to transmit a certain group of characters in the formation of a new organism. Each corresponding chromosome in different cells would represent the same group of characters while the different chromosomes of each cell would represent different groups of characters.

We have the right to assume that some chromosomes within the cell have greater influence in determining the characteristics of a new individual, otherwise they would all have equal power in this respect, which is inconceivable. Especially is this so when we see the great difference in the size of chromosomes within the same cell because there must be some purpose in this if the chromosomes are so important.

Naturally the larger chromosomes would represent larger groups of characters or else power to make the characters, which they determine, more prominent.

By a variation then in size of any one chromosome either the number or the intensity of the characters, which it represented, would be varied. If the chromosome is increased, its influence in the determination of the characteristics of the offspring would also be increased.

Knowing that the offspring from the same parents show considerable variation in their characteristics and having found that corresponding chromosomes of the same animal vary in size it seems probable that we have in this the cause of continuous variation.

According to this theory if an immature animal were mated with one of maturity, on account of the smaller size of its germ cell chromosomes (shown in the first portion of this paper) the immature germ cell would be overshadowed by the mature germ cell in the determination of the characteristics of the resulting offspring. In many cases this seems to be true though it is difficult to obtain trustworthy data.

V. Conclusion.

1. Germ cell chromosomes of very young nymphs are smaller than those of adults; this difference bears a direct relationship to the size of the germ cells.

2. As a result of either unequal growth or unequal division, corresponding chromosomes of different cells within the same individual vary in size.

3. The theory suggested by the observations in this paper is that the variation in the size of corresponding chromosomes of germ cells is the cause of continuous variation among animals.[1]

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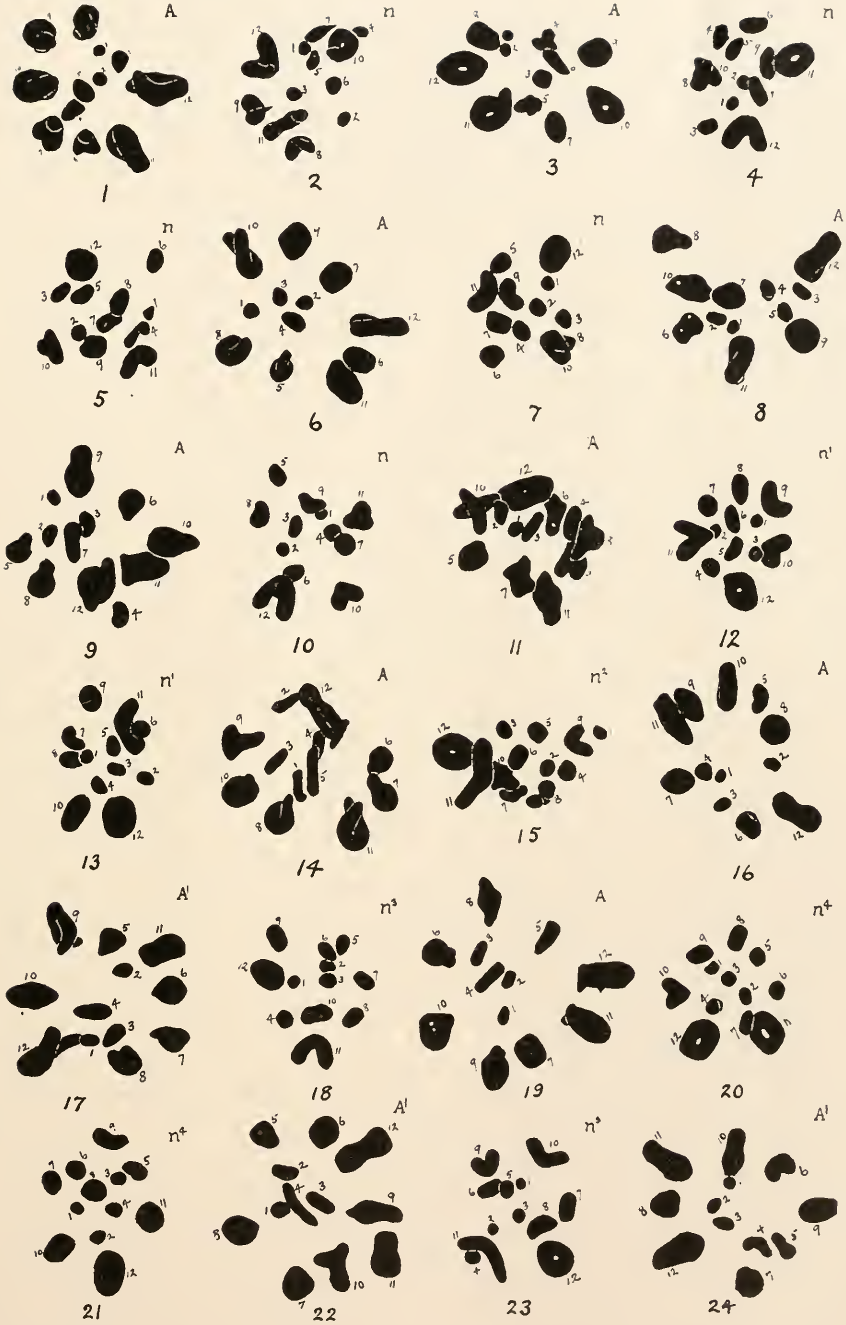
[1] The author is indebted to Dr. C. E. McClung for criticism.

PLATE I.

FIGS. 1 to 24 inclusive. Polar view of chromosomes in first spermatocyte metaphase. Drawings of this and the succeeding plates made with the aid of a camera lucida.

Figs. marked *A* are from adults, *A* and A^1 being from different animals. Figs. marked *n* are from young nymphs. n, n^1, n^2, n^3, n^4 being from different animals. Chromosomes are numbered according to their size in the cell beginning with the smallest. The difference in size between nymph and adult cell chromosomes is very striking. A comparison of corresponding chromosomes of different cells of the same animal shows the variation in size mentioned in this paper.

PLATE 1


A
1
n
2
A
3
n
4
n
5
A
6
n
7
A
8
A
9
n
10
A
11
n'
12
n'
13
A
14
n^2
15
A
16
A'
17
n^3
18
A
19
n^4
20
n^4
21
A'
22
n^5
23
A'
24

F. A. HARTMAN.

Plate II.

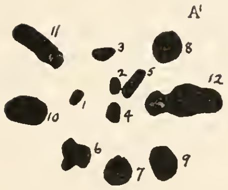
Figs. 25 to 32 inclusive. Polar views of chromosomes in first spermatocyte metaphase.

Figs. 33 to 47 inclusive. Polar views of first spermatocyte anaphase.

Scheme of lettering and numbering as in Plate I., n^5 and n^6 being from animals not represented in Plate I.

The greater size of the chromosomes of adults more pronounced than in Plate I.

In both metaphase and anaphase the variation in size, between corresponding chromosomes of the same animal, is evident.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3
8
2
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12
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4
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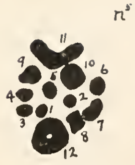
6
7
9

25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9
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6
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4
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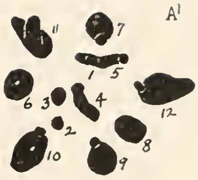
3
1
8
7
12

26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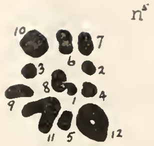
9

27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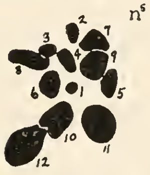
5
12

28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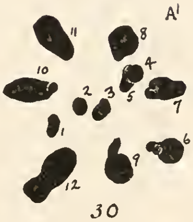
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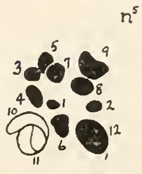
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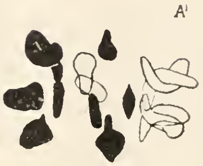
11
1

31



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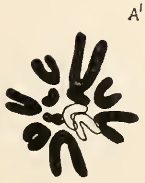

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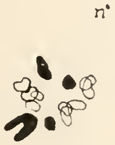

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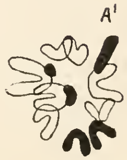

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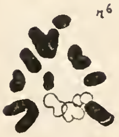

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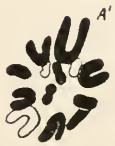

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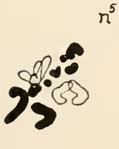

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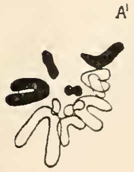

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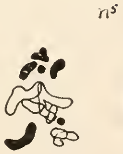

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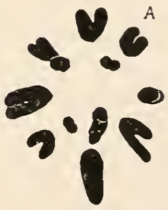

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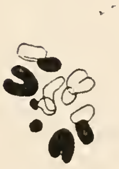

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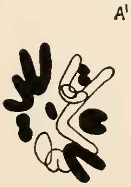

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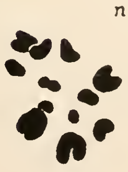

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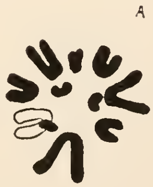

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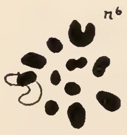

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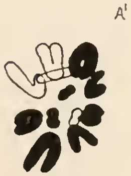


47

F. A. HARTM-N.

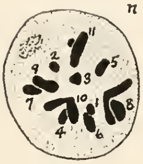
Plate III.

Figs. 56 to 63 inclusive. Polar views of the first spermatocyte metaphase, showing the variation in size of corresponding chromosomes of the same animal.

Figs. 56 and 57 are from one animal, Figs. 58 and 59 are from one animal, Figs. 60, 61, 62 and 63 are from one animal.

Figs. 48 to 55 inclusive. Polar views of the second spermatocyte metaphase. Figs. 48, 50, 53 and 55 are from young nymphs, 48, 50 and 55 being from the same animal. Figs. 49, 51, 52 and 54 are from adults, all but Fig. 52 being from the same animal.

The chromosomes and cells of the adults are larger than those of nymphs as in earlier stages. Corresponding chromosomes of the same animal also vary in size at this stage as well as in the next stage (anaphase) represented by Figs. 64 to 67 from the same animal.



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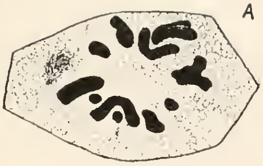

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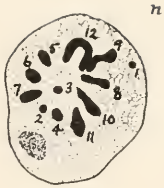

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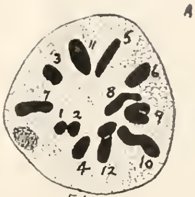

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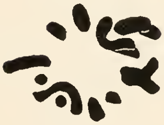

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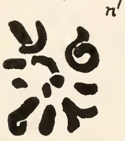

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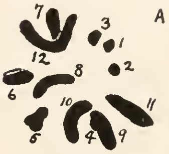

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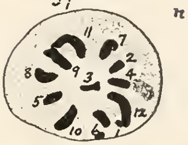

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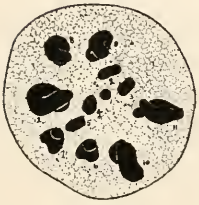

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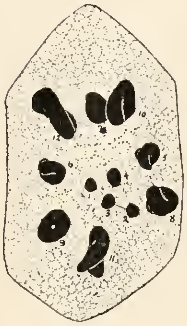

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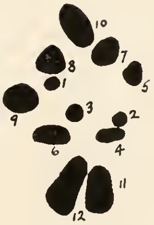

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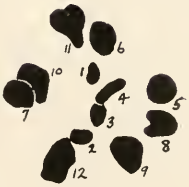

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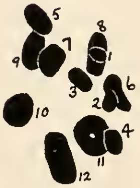

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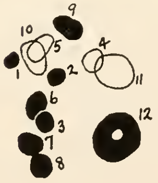

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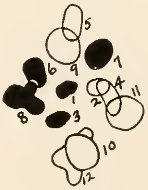

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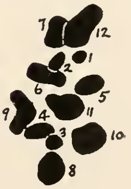

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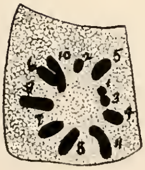

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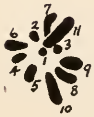

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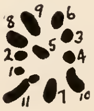

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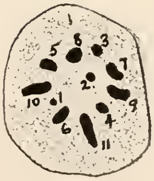

67

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PLATE IV.

FIGS. 68 to 74. Stages in the development of a single chromosome (*r*) during first spermatocyte prophase. The stain had been removed from all of the other chromosomes except the accessory.

FIGS. 75 to 82. Granules of the corresponding chromosome from other cells of the same animal.

Fig. 75. All granules divided.

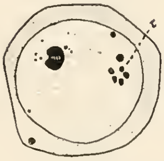
Figs. 71 and 72. Granules in the process of fusion.

The granules vary in size and number at similar stages.

FIGS. 85, 89, 90 and 91. First spermatocytes, showing chromosomes (*a*) dividing unequally.

FIGS. 86, 87 and 88. Other examples from the same stage, those in 88 being from the same cell.

FIGS. 83 and 84. Second spermatocytes, showing chromosomes dividing unequally.



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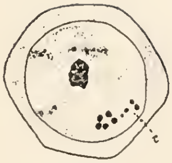

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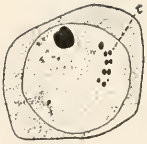

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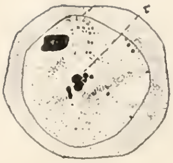

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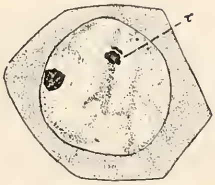

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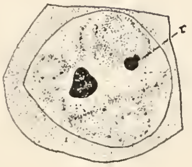

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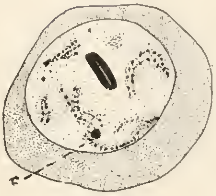

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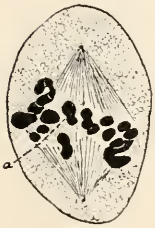

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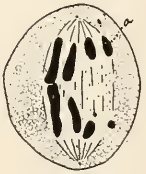

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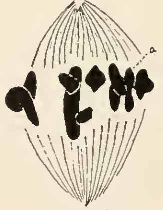

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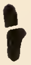

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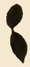

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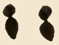

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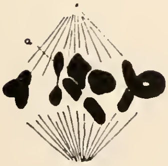

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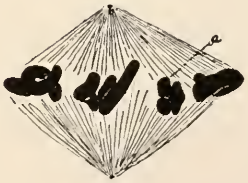

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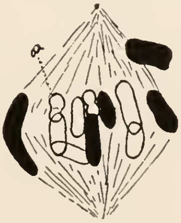

91

F. R. HARTMAN.

GIANT GERM CELLS IN THE GRASSHOPPER.

FRANK A. HARTMAN.

Giant somatic cells have often been described but so far as the available literature shows nothing but giant spermatids have been figured in the male germ cells of insects.[1] Mitotic figures of giant germ cells throw light upon the development of these abnormal spermatids.

One *Schistocerca* nymph about one third grown contained several giant cells in mitosis.

The earliest stage found was a secondary spermatogonium (Fig. 1) containing forty-six or more chromosomes. The chromosomes were so massed that it was difficult to determine the exact number, but the cell was equivalent to at least two ordinary cells (a typical spermatogonial cell contains twenty-three).

Two giant cells in first spermatocyte metaphase appeared; one of these did not show the chromosomes distinctly while the other (Fig. 2) contained twenty-nine chromosomes. As the number of chromosomes in the spermatocyte stages is reduced one half (to twelve) this giant cell corresponds to at least two normal cells.

An adult *Melanoplus* contained two giant first spermatocytes, each with twenty-four chromosomes, one was in late prophase and the other in metaphase.

A number of giant cells in first spermatocyte anaphase were found in the nymph already mentioned. Of these two are figured (Pl. I., Fig. 3, and Pl. II.). The cells were so large that they were divided into four pieces by sectioning. These pieces were easily distinguished by their relation to the surrounding cells. No doubt a few of the chromosomes were lost in sectioning, as one or two chromosomes were found detached from any cell.

There were fifty-four chromosomes passing toward one pole and forty-nine toward the other in the first of these cells.

---

[1] Paulmier, F. C., "The Spermatogenesis of *Anasa-tristis*," *Journal of Morphology*, XV.

In the second there were ninety-six on one side and eighty-one on the other. The first was equivalent to four or five cells and the last to eight cells. The chromosomes in these giant cells seem to act normally, that is, they divide and pass to the poles and maintain their individuality in every way, but the cell as a whole fails to divide.

In pathological mitosis the spindles are often multipolar and the number of chromosomes passing to each pole is then very unequal, while in these giant germ cells the mitotic figure is always bipolar and the number of chromosomes passing to each pole is nearly the same.

It may be that giant spermatozoa, formed from such cells as these just described, are the cause of monstrosities when they succeed in fertilizing an egg.

PLATE I.

FIG. 1. A giant secondary spermatogonium containing about forty-six chromosomes.

FIG. 2. An abnormal first spermatocyte with twenty-nine chromosomes.

FIG. 3. Chromosomes from a single first spermatocyte during anaphase. The cell of this figure and the cell represented in the succeeding plate were so large that they were each cut into four pieces in the process of sectioning. Fifty-four chromosomes toward one pole, forty-nine toward the other.

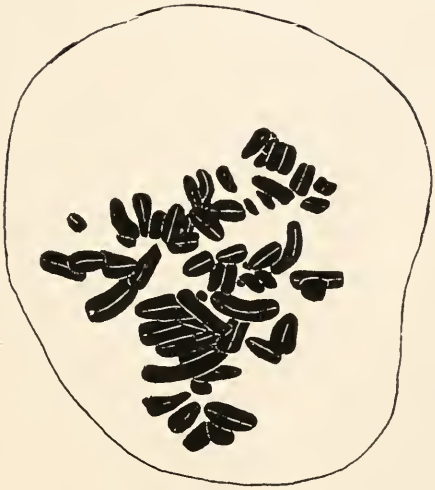

Fig. 1

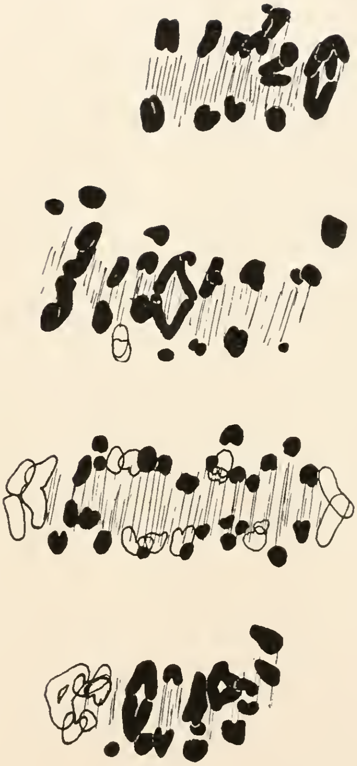

Fig. 3

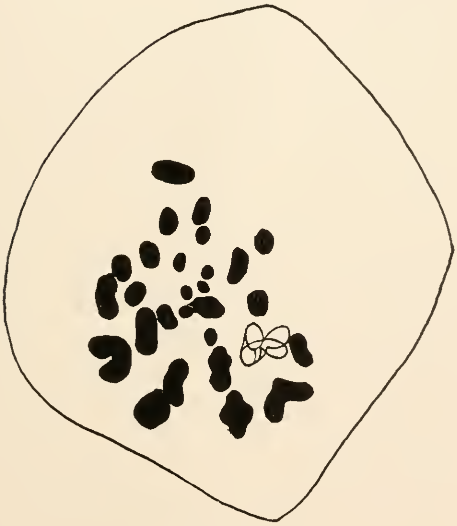

Fig. 2

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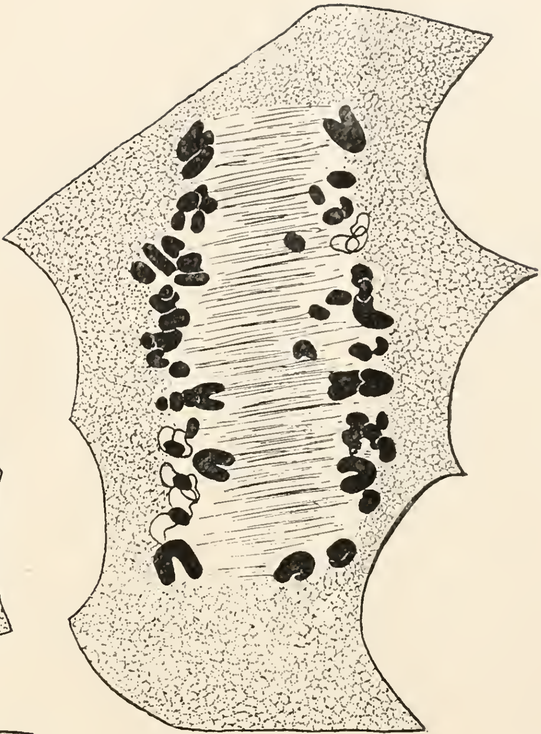
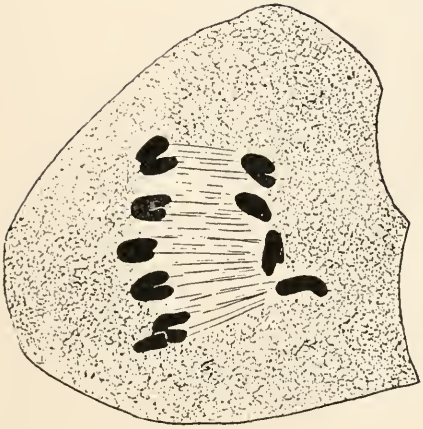
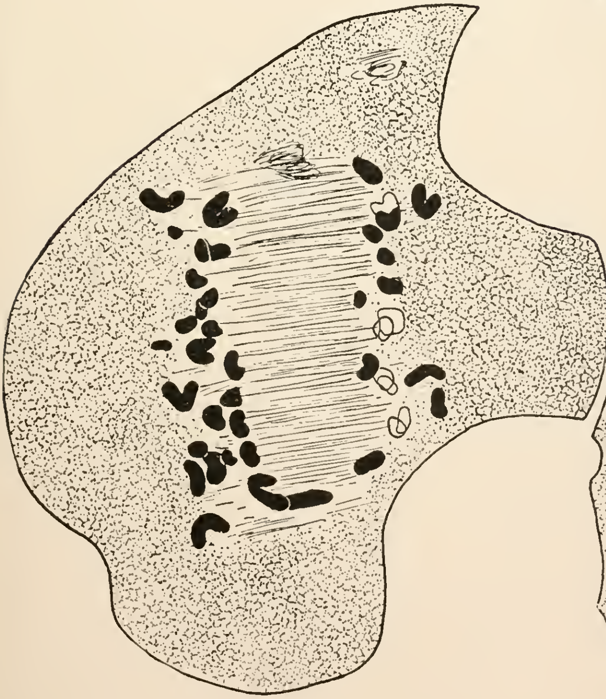
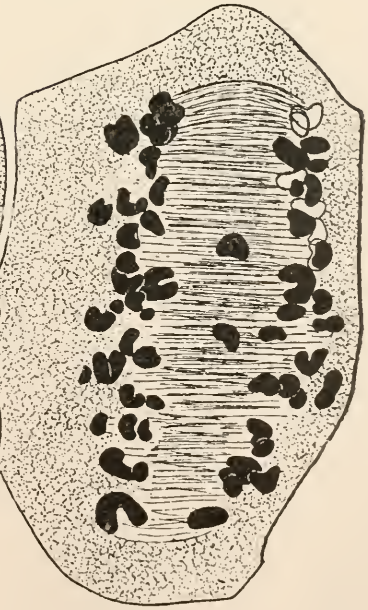
Plate II.

A giant first spermatocyte in anaphase with ninety-six chromosomes on one side and eighty-one on the other.









F. A. HARTMAN,

THE FERTILITY OF CECROPIA EGGS IN RELATION TO THE MATING PERIOD.

PHIL RAU AND NELLIE RAU,

SAINT LOUIS, MO.

Nature is lavish in her provision and wasteful in her economy. It is often difficult to tell where her reckless generosity ends and where the delicate limitations for the good of the species begin; in how far the individual shapes the race, or how far natural selection or other agencies eliminate the individuals in unnoticed millions in order to make the species the unit.

The *Cecropia* moth lays, on an average, about 300 eggs. After a free and natural mating, a considerable portion of these are found to be infertile. May it be that the prolific production thus tends to defeat itself by causing the great elimination?

We have also found[1] that a large per cent. of the females die still retaining a considerable number of their eggs, which it seems they have not sufficient strength or lease of life to deposit. This waste might also appear the direct result of the strain of producing such a large mass of ova.

Now we know[2] that the act of mating itself seems to curtail the life of the female by several hours, but even under these conditions of abbreviated time, oviposition is more nearly complete than when the individual remains unfertilized. Hence we see that while copulation itself works against the individual in curtailing its life, it works for the race in, in some way, increasing her power of bringing forth her young. In this far we find copulation, besides being a biological necessity, an actual economy in the production of a next generation.

Now when we consider these phenomena in connection with the fact that on an average 21 hours, or about 9 per cent. of the whole life of the insect, is normally spent in mating, the simple questions at once arise:

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[1] *Trans. Acad. Sci. St. Louis*, Vol. 20, p. 314.

[2] *Journ. Exp. Zoöl.*, Vol. 12, p. 199.

Does a longer period of copulation tend to insure the fertility of all the ova? or,

Does a larger number of ova require a longer period of mating in order to insure the fertility of all?

Is there a higher percentage of fertility among the first eggs deposited than among the last? (If so, the eggs retained after death may not be such a waste after all.)

At first thought one might reasonably assume that after a mating of normal duration,[1] practically all of the ova would be fertile. A moment's reflection tells us, however, that this is not the case anywhere in the animal or plant world. But there is nothing to tell us whether a shorter period of mating will result in a lower percentage of fertility. One might reasonably expect that in an abbreviated time the amount of sperm received into the seminal receptacle would be insufficient to fertilize the normal number of ova, and even more inadequate for an abnormally large number.

Whether or not such relationships exist, the following work will tend to show.

Material and Methods.

The 70 insects (35 ♂'s and 35 ♀'s) which supplied the following data were all isolated immediately upon leaving the cocoon, and as fast as mates emerged they were placed together, a single pair in a cage. Thus promiscuous mating was securely safeguarded against. All of the insects were comparatively young when mating began; none were over 24 hours old.

Frequently a few eggs would be deposited before mating began, but these were in all cases vigilantly destroyed. Early every morning the eggs from each cage were counted and placed in a vial with a cotton stopper and labeled; this comprised the oviposition of the preceding 24 hours.

The emerging caterpillars all died in their vials. Later these, as well as the empty egg-shells, were counted, and all the eggs which had not hatched were dissected to find whether they were infertile or whether they contained an embryo. In most cases all of the eggs that were shrivelled proved infertile and contained only a dried mass of yellow substance. It also usually happened that the smooth, round eggs contained a full-formed insect. There were a few exceptions in each class, however, and external appearance was not relied upon, but all eggs were dissected. Again a number of fertile eggs had openings of various sizes, sometimes too small to free the insect, and at other times perfectly cut, but the caterpillar it seems felt not inclined to leave the shell. These were in every case counted with those which normally emerged.

---

[1] Previous investigations have led us to conclude that, in this locality at least, the species is monogamous.

Fertility of Eggs and Duration of Mating.

The table shows the fertility of the eggs in relation to the time spent in copulo. All those which continued for about the normal length of time, 16 hours or more, behaved naturally and severed of their own accord; the others (3 to 15 hours) were for the most part artificially separated to ascertain the effect of the shortened period upon the fertility. The table is divided into first day's deposit, second day's deposit, etc., after mating.

An inspection of the columns reveals that there is absolutely no relation between the period of copulation and the fertility of the eggs.[1] We find that the insects which remained in copulo from 3 to 15 hours deposited just as large a per cent. of fertile eggs, and also as large actual numbers, as those which continued from 18 to 48 hours. Hence our present material at least would lead us to conclude that the first three hours are quite sufficient, and that a longer period is only a waste of valuable time and vitality. Especially does this seem distinctly disadvantageous to the species when we consider the number of eggs which the females produce but can not deposit in their short lives. If females which remain in copulo only 3 hours deposit as large a per cent. of fertile eggs as those which continue for 24 hours or more and afterward die without completely ovipositing, it would seem as though natural selection had missed its mark in not shortening the period of copulation and thereby lengthening the chance for oviposition.

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[1] The per cents of fertility were also calculated and tabulated for each individual of the entire lot, but these also showed no relationship whatsoever. Since the data are submitted it was thought superfluous to publish these per cents here.

♀ No.	Hours in Copulo.	1st Day's Deposit.				2d Day's Deposit				3d Day's Deposit.				4th Day's Deposit.				5th Day's Deposit.				6th Day's Deposit.				7th Day's Deposit.				Total.			
		T.	F.H.	F.U.	Inf.	T.	F.H.	F.U.	Inf.	T.	F.H.	F.U.	Inf.	T.	F.H.	F.U.	Inf.	T.	F.H.	F.U.	Inf.	T.	F.H.	F.U.	Inf.	T.	F.H.	F.U.	Inf.	De-posit.	F.H.	F.U.	Inf.
1	3	166	138	25	3	20	0	20	0	14	1	13	0	13	0	13	0	10	0	6	4	—	—	—	—	—	—	—	—	223	139	77	7
2	3	99	92	1	6	121	97	19	5	45	20	5	20	8	0	7	1	—	—	—	—	—	—	—	—	—	—	—	—	273	209	32	32
3	3	181	148	3	30	128	101	24	3	26	25	1	0	17	8	2	7	3	0	3	0	—	—	—	—	—	—	—	—	355	282	33	40
4	3½	205	169	25	11	83	7	73	3	44	1	0	43	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	332	177	98	57
5	4	189	158	2	29	?	?	?	?	?	?	?	?	?	?	?	?	42	30	7	5	6	4	0	2	—	—	—	—	237	192	9	36
6	4	80	62	2	16	96	69	19	8	62	6	47	9	?	?	?	?	1	0	0	1	—	—	—	—	—	—	—	—	239	137	68	34
7	4	140	132	0	8	120	118	1	1	6	4	2	0	2	1	1	0	1	1	0	0	—	—	—	—	—	—	—	—	269	256	4	9
8	5	89	86	0	3	62	3	58	1	27	8	16	3	?	?	?	?	1	0	1	0	—	—	—	—	—	—	—	—	179	97	75	7
9	12	20	14	0	6	68	53	2	13	95	93	1	1	21	18	1	2	10	7	1	2	—	—	—	—	—	—	—	—	214	185	5	24
10	2 + 8*	24	12	1	11	95	89	0	6	37	28	7	2	82	79	2	1	34	32	2	0	8	8	0	0	—	—	—	—	280	248	12	20
11	12	155	143	1	11	67	60	1	6	11	11	0	0	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	233	214	2	17
12	12	94	63	8	23	104	98	1	5	?	?	?	?	151	146	1	4	—	—	—	—	—	—	—	—	—	—	—	—	349	307	10	32
13	13	20	17	1	2	150	42	9	99	4	3	0	1	7	5	1	1	7	5	2	0	51	24	11	16	28	4	2	22	267	100	26	141
14	14	71	71	0	0	26	24	2	0	8	0	0	8	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	105	95	2	8
15	14	81	57	4	20	255	245	2	8	55	44	4	7	15	6	7	2	3	0	3	0	—	—	—	—	—	—	—	—	409	352	20	37
16	14	12	9	1	2	46	38	6	2	55	50	5	0	47	30	16	1	—	—	—	—	—	—	—	—	—	—	—	—	160	127	28	5
17	14	77	61	8	8	220	206	8	6	82	73	4	5	27	11	7	9	—	—	—	—	—	—	—	—	—	—	—	—	406	351	27	28
18	14	69	33	3	33	130	105	5	20	23	17	5	1	7	3	1	3	—	—	—	—	—	—	—	—	—	—	—	—	229	158	14	57
19	14	166	126	3	37	91	84	3	4	114	110	2	2	4	1	3	0	28	5	23	0	—	—	—	—	—	—	—	—	403	326	34	43
20	15	93	83	3	7	57	43	9	5	114	105	6	3	70	60	3	7	19	2	17	0	4	0	4	0	—	—	—	—	357	293	42	22
21	16	112	11	1	100	110	35	5	70	84	77	7	0	29	21	8	0	—	—	—	—	—	—	—	—	—	—	—	—	335	144	21	170
22	18	140	126	2	12	63	55	4	4	26	17	9	0	12	7	5	0	5	0	5	0	—	—	—	—	—	—	—	—	246	205	25	16
23	24	131	125	5	1	92	68	20	4	30	7	4	19	42	42	0	0	—	—	—	—	—	—	—	—	—	—	—	—	295	242	29	24
24	24	123	0	0	123	41	0	0	41	15	11	2	2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	179	11	2	166
25	24	158	5	17	136	32	20	9	3	27	16	10	1	16	13	2	1	10	5	5	0	—	—	—	—	—	—	—	—	243	59	43	141
26	24	114	77	17	20	82	80	2	0	12	11	0	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	208	168	19	21
27	24	190	135	51	4	74	40	32	2	31	15	16	0	33	21	9	3	—	—	—	—	—	—	—	—	—	—	—	—	328	211	108	9
28	24	111	0	0	111	121	0	4	117	28	7	0	21	2	1	0	1	—	—	—	—	—	—	—	—	—	—	—	—	262	8	4	250
29	24	56	0	0	56	41	0	0	41	25	0	0	25	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	122	0	0	122
30	24	165	5	6	154	48	18	3	27	9	5	2	2	?	?	?	?	3	0	0	3	—	—	—	—	—	—	—	—	225	28	11	186
31	48	77	45	26	6	3	3	0	0	3	3	0	0	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	80	48	26	6
32	?	36	18	1	17	3	2	0	1	58	0	0	58	49	0	0	49	47	0	0	47	53	0	0	53	3	0	0	3	249	20	1	228
33	?	240	184	31	25	2	0	2	0	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	242	184	33	25
34	?	78	55	10	13	81	74	5	2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	159	129	15	15
35	?	121	95	10	16	132	102	5	25	49	2	29	18	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	302	199	44	59

T. = total.

F.H. = fertile eggs that hatched.

F.U. = fertile eggs unhatched.

Inf. = infertile eggs.

* In copulo two hours one day and eight hours the next.

Furthermore, it is found that when the percentages of fertility of each day's deposit are calculated, in two thirds of the individuals the fertility increases as the days go by, while in only one third is there a decrease. Hence we are forced to admit another heavy loss; for this evidence would lead us to conclude that a large proportion of the eggs retained after death were also probably perfectly good.

In the material here considered we find also no correlation between this phenomenon and the long or short duration of copulation.

The table shows that in some of the cases of prolonged mating, the first day's deposit of eggs contained a great number which were infertile. Why this should be has not been ascertained. Being confident that all eggs laid previous to mating were destroyed, we are at a loss to explain why the first eggs, which one would think had the best opportunity of being fertilized, were otherwise. But a stranger fact still is that while female 29 was in copulo 24 hours, not one egg was fertile, and almost the same condition existed in females 24, 28 and 30.

One might think that those insects which carried an abnormally large number of eggs would have less chance for their complete fertilization. According to the data in our table, however, no such relation is found to exist. In fact the high percentage of fertility in many of these cases might suggest that the vigorous condition of the female contributed more toward insuring the fertility of the eggs than did the amount of sperm or the duration of copulation.

In all of the eggs in these experiments, then, 77 per cent. were fertile and 23 per cent. infertile. In the next to the last column of the table is given the number which partially developed but, from some unknown cause, died in the shell before hatching. These constitute 14.5 per cent. of the fertile eggs, or 11 per cent. of the whole number deposited after mating. All of the eggs were kept under identical conditions, so if 85.5 per cent. of the fertile ones hatched, the fact that these 14.5 per cent. did not hatch can not be attributed to environmental conditions.

Some of the eggs are laid in closely adhering clusters, and some are dropped singly. It was suspected for a time that the caterpillars had more difficulty in emerging from the clustered eggs and that many died in the attempt, but it was soon found that as many died in the shell in the loose as in the adhering eggs.

Hence the proportion which go to the wall even up to the time of emerging is large. We are reminded of Darwin's[1] statement that the ". . . geometrical tendency to increase must be checked by destruction at some period of life . . . and this period in the great majority of cases is an early one. . . . But if many eggs or young are destroyed, many must be produced or the species will become extinct." In our present material it would seem that nature, in making the attempt at providing so bountifully for the perpetuation of the species, has overstepped the limits of real economy by wasting so much unfinished material.

[1] "Origin of Species," 6th ed., p. 52, 1887.

THE LIFE HISTORY OF DESMOGNATHUS FUSCA.

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Although *Desmognathus fusca* ranks as one of the commonest of the American salamanders, at least in the eastern part of the United States, our published accounts of its life history are singularly meager and fragmentary. It is hoped, therefore, that the following account, culled from observations extending through several years during which this species has served as material for class work in anatomy, histology, embryology, and physiology in our laboratory courses at Smith College, as well as for several lines of individual research, may prove of some interest and even practical value to others who are engaged in the study of amphibian life. Incidentally, too, there is always the possibility that facts presented for their intrinsic interest may prove to have some broader biological significance, particularly in a field like that presented by the amphibians, in which there are involved, both phylogenetically and ontogenetically, so many environmental transitions which call for adaptive response.

Systematic Position and Specific Characters.

Desmognathus fusca is a representative of the family Desmognathidæ, which, together with the family Plethodontidæ comprises all of the American Urodeles now known to be lungless.[1] In common with the other members of these two families, *Desmognathus* possesses the naso-labial groove (Whipple, '06*a*) and lacks the ypsiloid cartilage (Whipple, '06*b*).

The following description of the external appearance of *Desmognathus fusca* is given by Morse ('04, p. 115): "*Desmognathus fusca fusca* Raf. Above dark, with a median dorsal band of lighter generally brown in color and specked with black. Below gray with black specks forming a marbling. . . . Very variable in color. Costal grooves 14. Length 4½ inches."

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[1] *Amblystoma opacum*, reported lungless by Lönnberg, has since been shown to possess fully developed, functional lungs (Whipple, '06*b*).

As pointed out by H. H. Wilder ('99), *Desmognathus fusca* may be conveniently distinguished from *Spelerpes bilineatus*, the other lungless species with which it is usually associated in habitat, by its more robust body, its general darker color, and less distinct markings. In *Desmognathus* the markings become more and more obscure with the increasing size and darkening color of the older adults. In many even of the smaller adult *Desmognathus* individuals the whole color becomes very dark and the ventral surface thickly mottled with pigment spots, while *Spelerpes bilineatus* remains practically unpigmented ventrally. The larvæ of the two species are, however, even more frequently confounded. Their differences have been clearly shown by

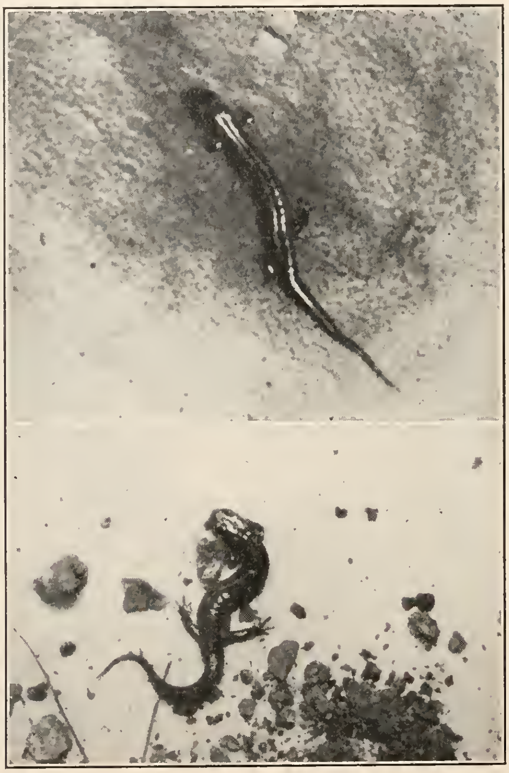


FIG. 1. Photographs of living adult *Desmognathus fusca*, in two characteristic attitudes, about one half natural size.

Wilder ('99). *Desmognathus fusca* larvæ undergo metamorphosis while still very small, from 28 to 30 mm. in length (although the duration of larval life is much longer than Wilder's estimate), while *Spelerpes bilineatus* may attain before metamorphosis a length of over 50 mm., greater indeed than the length of certain adult specimens. The *Desmognathus* larvæ may be distinguished from the younger *Spelerpes* larvæ by the proportionate size of the hind legs, which in *Desmognathus* are from the time of hatching much larger and stouter than the fore legs and are held when at rest at a considerable angle from the body, while the hind legs of the small *Spelerpes* larvæ are slender and scarcely exceed the fore legs in length. The digits of both fore and hind feet of *Spelerpes* larvæ of all sizes are attenuated, and the feet never have the robust appearance of those of *Desmognathus* larvæ.

Morse ('04) says of *Desmognathus fusca:* "The larvæ attain a length of three inches before becoming mature, and inhabit springs and small bodies of water. They are brown with black dots above, lighter below, retaining also the black dotting. The gills are short and inconspicuous. Sometimes the black is dotted here and there with livid specks of red, but this disappears in the adult condition." This is undoubtedly a case of incorrect identification as the description does not correspond to *Desmognathus fusca* larvæ in the matter of size, color, or habitat. I am able to refute the identification with considerable positiveness for the reason that my conclusions concerning every stage of the life history of this species have been corroborated by careful study for several successive years of material obtained from a much circumscribed little stream in which *Desmognathus fusca* is the only salamander that occurs.

Habitat.

As compared with other Urodeles, *Desmognathus fusca* may well rank as one of the more terrestrial. Its lungless condition is in itself an indication of its terrestrial habitat; for, as pointed out in a previous paper (Whipple, '06*b*), amphibian lungs serve an important hydrostatic function, so that with the exception of certain lungless forms such as *Spelerpes ruber*, which have secondarily adapted themselves perfectly to aquatic life at the bottom of the water, the lungless condition unfits an amphibian for a prolonged stay in the water. If further evidence on this point is desired one has only to drop living specimens of *Diemyctylus viridescens* and *Desmognathus fusca* into a deep aquarium and compare their behavior!

The lungless salamanders show, however, varying grades of environmental adaptation, from *Spelerpes ruber* above cited as a perfectly aquatic form, to *Plethodon cinereus*, which is completely terrestrial even during its larval life, while the extreme is reached in the case of *Autodax lugubris*, reported by Ritter and Miller ('99) to be practically arboreal throughout its entire life.

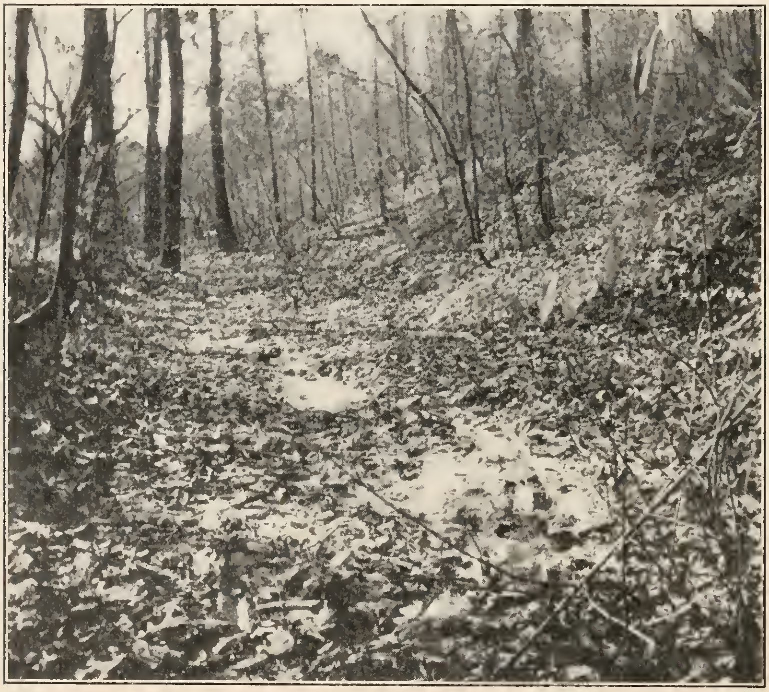


FIG. 2. Photograph of a typical habitat of *Desmognathus fusca*, as it appears in late autumn, when the water in the little brook is for the most part covered with fallen leaves, which serve as a protection to both the larvæ and the adults.

Desmognathus fusca is terrestrial to the extent that it lives in and upon moist earth, where it mates and lays its eggs, and where the first few days of the larval life are spent. The young larvæ, however, soon reach shallow running water where they remain for nearly a year, after which they come out of the water to live the terrestrial adult life of the species. The presence of aquatic as well as terrestrial insect forms in the stomachs of adult *Desmognathus* shows, however, that even the adults are by no means absolutely terrestrial, but, so far as my own observations and those reported by others go, the adults confine their aquatic excursions to very shallow water, and they are seldom found in the water at all unless driven there.

Apparently, the ideal environmental conditions for *Desmognathus fusca*, as deduced from a study of those localities in which they occur in the greatest abundance, are those afforded by the banks of well shaded streams of shallow, perennially running water. In such places they may usually be found under every stick and stone and fallen log at or below the water level, while the soft earth is riddled with burrows, often, it is true, the work of earthworms or of other animals, but utilized as convenient lurking places for *Desmognathus;* for the adult *Desmognathus* is, in the day time, at least, thigmotropic, and will even take refuge in a bottle left lying on the surface of the soil exposed to the light, usually orienting the body with the head toward the mouth of the bottle. *Desmognathus* has the power to make its own burrows, as it will demonstrate, sooner or later, when isolated in a terrarium which affords no other suitable means of protection. In one case in particular which came under my observation, two individuals were accidentally set aside in a terrarium which was supposed to contain no specimens. Over a year afterwards it was found that these animals were still living in this terrarium under almost perfectly dry conditions. In the soil were many well formed burrows, within which the specimens, which were kept for some time after they were discovered, remained, often completely concealed, but at times with the head protruding from the entrance to the burrow.

Under natural conditions the crevices and burrows inhabited by *Desmognathus* are, of course, very moist, and I have usually been able to demonstrate, by following them carefully through their windings and branchings, a connection with the water of then eighboring brook.

The part which the shaded condition plays in the habitat of *Desmognathus* is undoubtedly a somewhat complicated one, possibly determining conditions for the necessary food supply, as well as helping to maintain a moist condition of the soil, supplying an abundant and annually replenished loose earth from fallen leaves and twigs; those leaves, also, which fall into the water furnish lurking places in which the larvæ take refuge and seek their food. The element of close proximity to running water in the habitat of *Desmognathus* is certainly not necessary to the immediate physiological demands of the adult, but is incident rather to the aquatic nature of the larval life, which makes necessary not only easy access to water after hatching, but also requires that the supply of water shall be perennial, since each year the newly hatched larvæ reach the water at about the time when the brood of the previous summer leave the water as very small adults. Upon the other hand, these small adults upon leaving the water find themselves in the midst of the proper external conditions for their whole adult life, including mating and egg laying, and therefore, so far as is known, there is no tendency whatever to annual migrations such as has been shown to occur in *Amblystoma punctatum*, for example (Wright, '08).

MATING HABITS.

In his valuable discussion of the breeding habits of amphibians, Smith ('07) has pointed out an interesting gradation in methods of fertilization from the lavish method of typical aquatic fertilization possessed by *Cryptobranchus*, in which the unfertilized eggs are expelled into the water in large numbers, to take their chances at being fertilized by the abundant spermatic fluid expelled by the male in their vicinity, to the opposite extreme of typical internal fertilization without spermatophores, which the *Apoda* have been shown to have acquired as an adaptation to their completely terrestrial existence. Between these two extremes lie such forms of fertilization as that of the *Amblysstoma punctatum*, in which the spermatozoa are not poured freely into the water, but are enclosed within spermatophores which the male, stimulated by the presence of the ripe or nearly ripe female, deposits in considerable numbers, leaving with the female, however, the whole responsibility for the entrance of one of these into her cloaca (Wright and Allen, '09); and the still more certain method of the various *Tritons*, in which the male uses special devices, such as clasping, to ensure the entrance into the cloaca of one of the small number of spermatophores, thus making certain the fertilization of a far larger proportion of the reduced number of eggs. Smith ('07) points out that in the increasing economy in the amount of seminal fluid shown by such mating habits as those of the *Amblystoma* and *Triton* we see, as an incidental result, a preparation for possible terrestrial life.

In *Desmognathus fusca* we see a form which has availed itself of this, as well as of other possibilities of adaptation to terrestrial existence; for, although the larvæ retain the typical aquatic nature of amphibians, the habit of a periodical return to the water for the mating and egg laying which an aquatic larval life usually involves has been abandoned, and both of these functions are performed by the adults without the inconvenience of leaving their terrestrial abode. My information concerning the actual act of mating is drawn from a single observation. Since, however, both Kingsbury ('02) and Hilton ('09) state that nothing definite seems to have been published upon this point, I feel warranted in giving a full report of this single observation.

On the evening of May 13, 1908, I isolated in a small terrarium a large male, and a female through the abdominal wall of which large eggs could be seen. It was discovered the next morning, however, that another smaller male was also present in the terrarium, probably having been carried over unobserved in transferring some wet leaves. On the following morning, May 14, the female and this smaller male were found lying upon the earth under some wet leaves, the ventral surfaces of the bodies in contact. They reacted so quickly, however, to the disturbance of the leaves that beyond this very hasty observation as to their general position I can state nothing definite as to methods of clasping or exact regions of contact. Protruding from the cloaca of the female was a yellowish, semi-fluid mass which was found upon examination to be a spermatophore of very soft consistency.

When placed in a drop of water upon a slide, the spermatophore was found to contain large numbers of tightly coiled spermatozoa (Fig. 3, *x*), which, however, in a few minutes began to uncoil, so that in the course of half an hour the majority were uncoiled or uncoiling. Some few, however, were still coiled seven hours later. From time to time some of the spermatozoa were seen to be coiling up again, a condition which was probably induced, as was shown by a subsequent study of spermatozoa taken from the ducts of living males, by partial drying of the preparation. All of these movements were somewhat jerky and mechanical, and none of the free swimming movements such as the living spermatozoa exibit within the ducts of the male were to be seen. It must be noted, however, that the conditions were not perfectly normal, since the spermatophore, obviously not intended for an aquatic medium, was placed in water for examination. In the case of another spermatophore, found in the cloaca of a similarly isolated female, and examined microscopically without the

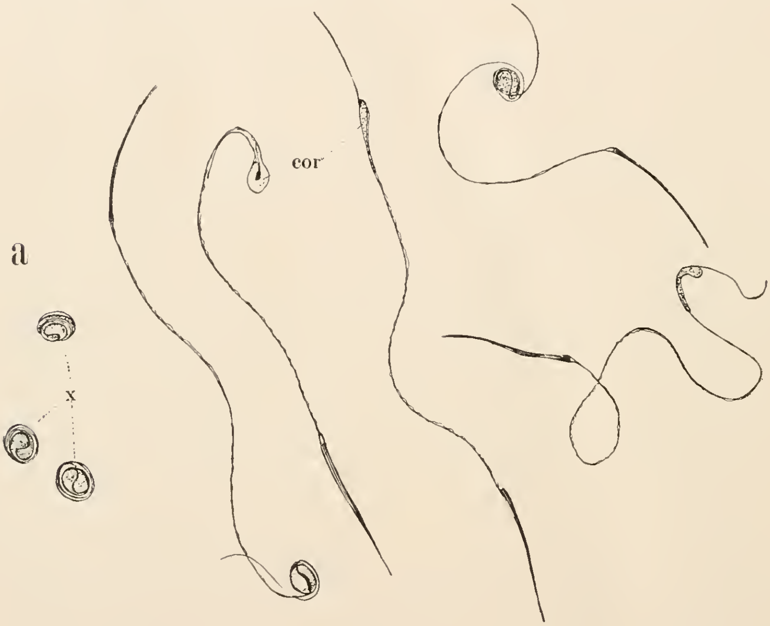


Fig. 3. Spermatozoa of *Desmognathus fusca*, showing method of coiling; *cor*, the mass or corpuscle about which the spermatozoa coil; *x*, spermatozoa coiled as found in the spermatophore. Based upon drawings with Abbé camera. × 290.

application of water, the spermatozoa were coiled and exibited none of the uncoiling movements. In this case the spermatophore was of a much firmer consistency, which, as no male was found with the female, may have been due to the longer lapse of time after its transfer to the cloaca of the female.

Kingsbury ('02) concluded from the presence of spermatozoa in the cloaca of the females captured at all seasons of the year, that the fertilization is internal, and further inferred from the condition of the ducts and cloaca of the male at various seasons, that the fertilization takes place not only in the spring but possibly also in the fall. The whole subject is one which demands a careful series of observations and experiments, which are, however, rendered somewhat difficult, as pointed out by Kingsbury, by the retired nocturnal habits of the animal. The above report is at least sufficient to show that the mating in this species takes place under terrestrial conditions and that the fertilization is an internal one, facts which are quite in accord with the general adaptation to terrestrial life shown by *Desmognathus*.

In my examination of spermatozoa I have invariably noted a certain appearance of which I have as yet found no mention in the literature upon the subject. Spermatozoa taken in a living and active condition from the ductus deferentia of a decapitated male, as well as those removed from the cloaca of the female, may be seen to constantly coil and uncoil, assuming in the process such appearances as are shown in Fig. 3. In uncoiling, however, there is gradually revealed an elongated or oval mass (*cor*), apparently of semifluid consistency and of a translucent material, which seems to form the core about which the coiling takes place and which is enclosed in the last final loop of the tail as it uncoils. As spermatozoa within the tubules of the testis do not possess this structure, it is apparently acquired in the passage from the testis into the duct, and is very likely a secretion of mucous nature derived from certain specialized cells. Its nature and source, however, as well as its function require further investigation. If it is peculiar to *Desmognathus*, it may well be an adaptation to the prolonged period which must often elapse between the introduction of the spermatozoa into the spermathecæ of the female and the actual fertilization of the eggs, and is one of the many devices which it is necessary for a terrestrial form to develop to resist the drying action of the air.

EGG LAYING HABITS.

Concerning the egg laying habits of *Desmognathus fusca* we have several published reports. According to Hilton ('09), the eggs are found most abundantly from the last of June to the middle or last of July. Kingsbury ('02) reports eggs of *Desmognathus* found at Ithaca during July and August, and refers to the statement of Sherwood ('95) that they have been found from July to October. Reed and Wright ('09) give July as the time of maximum egg laying. In western Massachusetts, in the immediate vicinity of Northampton I have found eggs as early as the eleventh of June and as late as the twenty-fourth of September, although, since in the latter instance the eggs were just at the point of hatching, they were probably deposited during the latter part of August. As reported by H. H. Wilder ('04), eggs have been deposited by individuals in captivity in the Smith College laboratory as early as June first. It is thus safe to state that the egg-laying period extends at least from the first of June to the last of August and possibly through September. As suggested by Hilton, temperature and humidity may have much to do with determining the exact time, and it should be further noted in this connection that the latest brood of my own observations, that of September 24, 1907, occurred at the end of a singularly cold summer, and may have been belated from that cause.

The eggs are laid in a small batch consisting usually of two masses and numbering about 20 in all (15–20 according to Hilton), a number which corresponds in general also to the count of the ripe eggs found in the ovaries of a large number of females which I have examined for purposes of comparison. The total number is practically constant, being 10 or 11 in each ovary. A few show a smaller number or a greater inequality, such as the case of 9 in the left and 5 in the right ovary, or of 13 in the left and 4 in the right ovary.

The small number of eggs deposited at a time as compared, for example, with several hundred reported for *Cryptobranchus alleghenienensis* (Smith, '06, '07 and '12), 130–225 for *Amblystoma* *punctatum* (Wright and Allen, '09), 108 for *Diemyctylus viridescens* (Jordan, '91), and 150 for *Amphiuma means* (Hay, '88 and '90), shows a striking correlation with the far greater certainty of fertilization which is insured by the direct deposit of the spermatophore in the cloaca of the female; the small number of eggs is also undoubtedly correlated with the superior chances for successful development afforded by the large size of the egg (about 3.5 mm. in diameter), and the maternal care during development.

The eggs are laid in a little moist hollow or cavity, often of accidental occurrence, in just such places, in fact, as those in which the adult *Desmognathus* is usually found. I have observed that the female in captivity will sometimes excavate in the soft earth underneath a stone or clump of moss a cavity sufficiently large to accommodate her body in a coiled position, and in this cavity will deposit her eggs. In nature I have found eggs under a mere covering of moss or a little decaying stick. Hilton ('09) reports that they are found also at a depth of as much as four feet below the surface. They seem never to be deposited more than two or three feet from the edge of the water, or more than a few inches above its level in the neighboring brook, into which the newly hatched larvæ are destined to find their way.

So far as my own observations and those reported by others go, the eggs are laid during the night or in the early morning, as would indeed be expected from the nocturnal habits of the species. No cases have been reported of the prolongation of the process of egg laying beyond the single night during which, apparently, all the eggs produced by a given female for one season are deposited.

The eggs are always found guarded by a female, undoubtedly the mother. She usually so places herself among them as to bring practically all of the eggs in contact with her body, which often extends through the mass of eggs and is frequently bent sharply upon itself as if the better to surround and protect them. When under observation, as in a terrarium, the mother frequently leaves the eggs when disturbed, always retreating through the same exit from the nest. After having been separated from the eggs, however, as may occur in making a transfer from out of doors to the laboratory, the mother goes back to them again, even though the nest and all of its surroundings may have been reconstructed. I have never had the opportunity to further test the sense of ownership of eggs in a mother by exchanging the eggs of two individuals, but the experiment would certainly be an interesting one.

The function of the mother in incubating the eggs is probably mainly that of insuring the proper degree of moisture, since eggs when removed from the mother and kept moist undergo normal development. It is possible, also, that she may guard them from inundation; for the first eight or ten days eggs will develop normally, even though quite immersed in water, but during the later development a short immersion in water kills them, apparently through a rapid change in osmotic pressure between the cells of the embryo and the fluid which by this time surrounds the embryo within the protective envelopes.

I have no evidence that the habit of eating the eggs, such as Smith ('07) found in the case of *Cryptobranchus*, is ever indulged in by *Desmognathus*, although, as will be shown below, this species is by no means to be exempted from the charge of cannibalism.

The Eggs.

The egg membranes, already very carefully described by previous writers, are three in number, the outer one being continued into a stalk which connects the eggs with the others, and is thus a common membrane for the whole mass. Smith, ('12) in comparing the egg strings of *Cryptobranchus allegheniensis* with the arrangement of the eggs of other Urodeles, suggests the probability that the most closely related egg arrangement is such as is shown by the stalked egg enclosures of *Desmognathus* rather than in the jelly-enclosed masses of certain other forms. The accompanying drawings (Fig. 4) of the two egg masses of a batch of eggs studied by me suggest the probability of the derivation of their arrangement from a more primitive rosary arrangement, such as *Cryptobranchus* possesses. A few eggs are still seen in the main axis of the bunch, but the majority have pushed out to one side, as if through an excess of lateral pressure brought to bear upon the outer envelope from within. Each egg has thus acquired a short stalk which connects it with the other eggs of the bunch. Whether this is a change which takes place in each individual case during the descent of the eggs through the oviduct and the subsequent reaction upon exposure to air, or whether this is a form of egg string which has been derived phylogenetically from some ancestor in which the egg string was in the rosary form, I am unable to say. The stalk of each egg becomes eventually very much twisted, probably a secondary condition brought about by the movements of the mother among the eggs while brooding them; this twisting tends to increase the length and diminish the diameter of the stalks, thus crowding the points of their mutual attachment nearer together and emphasizing the relationship expressed by H. H. Wilder's comparison of the whole mass of eggs to a bunch of toy balloons.

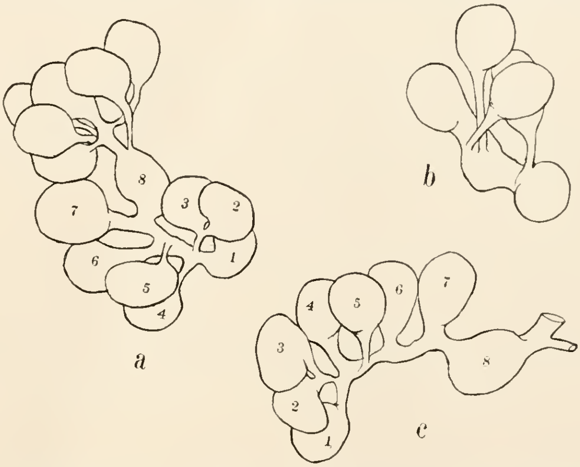


Fig. 4. Sketches of egg capsules of *Desmognathus fusca* after the larvæ have hatched; (*a*) and (*b*), the two masses of the same batch of eggs as is shown in Fig. 6; (*c*) a view of the egg capsules numbered 1–8 of (*a*), so arranged as to show their probable sequence with relation to the main stalk of the mass, and suggesting the derivation of the form of egg mass from a more primitive rosary form. Approximately twice life size.

The eggs themselves are creamy white, unpigmented spheres, each measuring from 3.5 to 4 mm. in diameter. This large size provides, of course, for a more advanced stage of development at the time of hatching and thus insures the preservation of a very large percentage of the brood. The large size of the eggs, the certainty of fertilization which the internal method insures, the well-sheltered position in which the eggs are placed, and the maternal care during development are thus all conditions which compensate for the very small number of eggs.

Within the protective envelopes, the eggs orient themselves, after the manner of amphibian eggs, with the animal pole above· they continue this orientation until the embryos reach a sufficiently active stage to introduce other factors into the determination of their position in the egg.

Embryonal Development.

Without attempting to enter into the details of the embryology of *Desmognathus*, certain general features of the course of development of the egg are in place in an account of the life history of the species.

The early development of *Desmognathus* eggs has been worked out by both H. H. Wilder ('04) and Hilton ('04 and '09). The segmentation is found to be holoblastic, although, as demonstrated by Hilton, the large size of the yolk mass prevents the complete division internally until after the early blastula stage is reached, thus placing the egg on the border line between the holoblastic and meroblastic types, while throughout its entire development the appearance of the embryo as it lies at first upon, and later coiled around, the large mass of yolk cells, so strongly suggests the condition found in meroblastic eggs that one instinctively speaks of "embryo and yolk" (Fig. 5). This resemblance is emphasized by the fact that the conspicuous capillary network which early develops on the surface of the mass of yolk strongly suggests the yolk circulation of meroblastic forms. Wilder ('04) noted the correspondence externally of certain of these blood vessels with the lateral cutaneous and median abdominal veins of the adult, and on this ground did not consider them the homologues of the vitelline veins of meroblastic forms. Piersol ('09), on the other hand, describes a similar meshwork of blood vessels in the splanchnic mesoblast of the embryo of *Plethodon cinereus erythronotus* as a vitelline circulation, and as sections of *Desmognathus* embryos show the blood vessels in question to be similarly situated in the splanchnopleure (Fig. 11, *vbv*), there would seem to be no reason why they should not be considered the homologues of a true vitelline circulation.

The segmentation process results in the distribution of the yolk granules to the cells of the blastula, and there early appears, according to Hilton ('09), a differentiation of the cells into those containing large and those containing small yolk granules, the latter arranging themselves in a superficial layer surrounding the former, which form the whole central portion of the mass. Wilder ('04) found that the late blastula stage is reached in about three days, while Hilton reports the formation of the yolk plug stage in about forty hours from the time the eggs are laid. This discrepancy may, however, be due to an individual difference in the rate of development, since Wilder found existing simultaneously in the same batch of eggs, four-, eight-, and sixteen-cell stages.

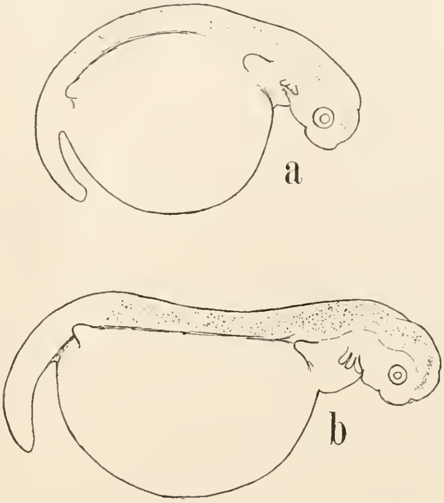


FIG. 5. Two embryos of *Desmognathus fusca* removed from two of the same batch of eggs after 16 days of development, showing a difference in the rate of development. Drawn with Abbé camera. × 9.

By the eleventh day both the anterior and the posterior ends of the embryo are well lifted above the surface of the yolk, and the dorsal region of the body appears as a narrow welt or ridge stretching between them. By the thirteenth or fourteenth day the embryo begins to make spontaneous movements, forcibly bending the free anterior and posterior ends of the body to right and left. By this time the limb buds have appeared, and the dorsal surface of the embryo assumes a decidedly grayish hue, owing to the beginning of pigmentation, which rapidly increases as development goes on, and takes the form of a decided pattern by the twenty-fourth day.

External gill bushes may be readily distinguished as early as the sixteenth day. On the twenty-fifth day, however, neither the mouth nor the gill slits have opened to the exterior. On the thirtieth day the mouth has opened by two lateral slits but is still closed in the middle region. At this time, also, the two most anterior gill slits upon each side are open, the more anterior being just posterior to the gular fold.

A considerable accumulation of liquid early appears about the embryo, and continues to increase in amount, distending the egg envelopes, which are thus placed under a constantly increasing tension. From this cause it becomes increasingly easier to remove the embryo from the egg membranes, until by the eighteenth day a puncture of the membranes is followed by a rapid expulsion of the embryo through the force of the escaping fluid. This is undoubtedly a preparation for the easy rupture of the membranes and escape of the embryo when the time for hatching arrives, the fluid serving meanwhile to equalize the external pressure upon the delicate embryonal tissues and prevent injury to them.

Thirty days is the longest time for which the continuous development of any batch of eggs has been reported (H. H. Wilder, '99), so that as to the exact duration of the period of development within the egg I have very limited data. It is seldom that one knows the exact date when a given batch of eggs is deposited, or, knowing this date, can carry on the observations successfully under artificial conditions to the time of hatching. Hilton states that the rapidity of development depends upon humidity and temperature, but gives no data whatever concerning the duration of the whole period. Wilder describes the thirty-day embryo above referred to as a fully developed larva in all respects except the still large yolk mass. "The pigmentation was complete, the external gills fully developed, and the feet had the full number of distinct toes (4 anteriorly and 5 posteriorly)." This embryo was 13 mm. in length, 2 mm. shorter than the shortest newly hatched larva which I have measured, and Wilder estimated that its supply of yolk might indicate a continuation of development within the egg for several days longer. Thus the length of the entire period might be estimated at approximately 5 weeks.

The length of the period of development within the egg varies, however, even in the same batch of eggs, since the hatching process sometimes continues for four or five days. This difference in the time of hatching may possibly be due to the element of chance in the occurrence of sufficient friction to rupture the membranes; since, however, the newly hatched larvæ have, so far as my observations go, reached the same stage of development, though differing slightly in size, it is more probable that this difference in the date of hatching of members of the same brood is due to an actual difference in the rate of development. The difference which Wilder found in the segmentation stages of eggs of the same brood, which might be logically accounted for by a slight difference in the exact moment of fertilization, would in itself be insufficient to account for so great a difference as four or five days in the time of hatching, or for the decided difference which I have observed in the development of embryos taken simultaneously from the same batch of eggs during the latter part of the embryonal period (Fig. 5, *a* and *b*). Piersol ('09) noted a similar difference in the rate of development and consequent time of hatching in the eggs of *Plethodon cinereus erythronotus*, and attributed it to a difference in the accessibility to a supply of oxygen, the eggs on the outside of the mass having the better chance. In the case of *Desmognathus*, however, the eggs are so arranged and so frequently shifted in relative position by the movements of the mother, that it is hardly possible that they differ greatly in their access to either moisture or oxygen supply. Hilton noticed that the smaller eggs of the mass usually develop more rapidly, a condition which might readily result during the early stages from the smaller mass of inert yolk material upon which to expend the energy of cell division, and possibly in the later stages from the larger ratio of surface for absorption, to mass.

Hatching, and the Terrestrial Larval Period.

Since the development and hatching of the egg take place under terrestrial conditions, it follows that there must be a terrestrial larval period between the time of hatching and the time when the larvæ reach the water and enter upon their aquatic larval life. Concerning this terrestrial larval life of *Desmognathus* and the important changes which the transition from terrestrial to aquatic conditions naturally involves, there is, so far as I know, no published account. For this reason I shall give somewhat in detail an account of a case of the hatching and subsequent development of a brood of *Desmognathus* larvæ which came under my observation.

The batch of eggs in question were found on September 24. They were found in the usual sort of location, under a decaying stick about three feet from the edge of the water of a certain shallow brook in which *Desmognathus* abounds. A large female was, as is usually the case, coiled about the eggs, while nearby under the same stick was a smaller adult, which proved to be a male. As it is not uncommon to find two or more individuals under the same stick or stone, this fact has probably no significance. The eggs contained large and very active embryos, each still distended with a conspicuous mass of whitish yolk.

As I had not the implements at hand for removing a sufficient mass of the loose earth underneath the nest to avoid disturbing the eggs, I carefully replaced the stick and left the eggs and the adult undisturbed, intending to return immediately and remove them. This a heavy shower prevented, and the nest was not revisited until the next morning. The female was found under the stick two or three inches from the eggs, which had already begun to hatch. She was in a rather poorly defined burrow and was headed away from the eggs. The whole family (mother with the hatched larvæ and unhatched eggs) was taken up upon a mass of soil included in a radius of about five inches and several inches deep, and was carefully transferred to the laboratory. The mother made no attempt to escape and during the journey returned to the eggs and placed herself among them (Fig. 6). Three larvæ and fifteen unhatched eggs were counted at this time; it is probable that two larvæ had already escaped from the nest, however, as a subsequent examination of the egg envelopes after all had hatched showed the entire number of eggs to have been twenty. The larvæ were very active and crawled about over the moist body of the mother; when disturbed they made quick jerky jumps among the loose debris of pine needles and decaying leaves upon which the eggs were lying. Two of the larvæ were killed at this time and were found to measure 15 and 15.5 mm. respectively (stage *A* of the subsequent description).

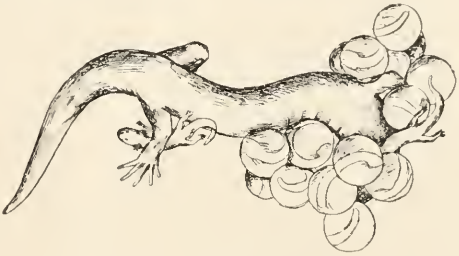


FIG. 6. Sketch of an adult female *Desmognathus fusca* with batch of hatching eggs. Drawn Sept. 25, 1907, by H. H. Wilder. Approximately life size.

The whole mass of soil was placed undisturbed in a small round glass terrarium, and the eggs, the remaining larva, and the adult were covered with some large wet leaves. A shallow crystallizing dish, filled with water and buried to the brim in the dirt at the bottom of the terrarium, was provided to take the place of the brook into which, if left undisturbed out of doors, the larvæ would presumably have found their way. Water was added to make the soil as thoroughly moist as in the natural habitat.

For five days the hatching continued at the rate of two or three a day. During this time the behavior of the mother and the young was frequently observed. In the course of the first 24 hours the position of the egg mass was entirely changed, probably owing to the gradual shifting due to the movements of the mother. After two days the mother no longer remained in contact with the eggs, but she stayed nearby in a burrow which she had made in the loose soil; she was usually found lying in this with her head very near the hatching eggs. Little by little the newly hatched larvæ would stray from the nest, and would be seen, often in groups of two or three, disappearing into deeper and moister crevices in the loose soil. Often one or more would be seen in contact with the mother in her burrow. During the last two days of the hatching period, the mother was still deeper in her burrow, and although she may have returned to her nest unobserved, she never was found there. The larvæ, also, were vanishing, so that by the time the last of them hatched, only eight were visible in the nest.

Later, on October 1, search was made to learn what had become of the larvæ. Several were found in the loose mass of decaying leaves, in burrows which had been made by the mother, or in natural crevices in the loose soil. These were in groups of from two to four with the bodies in close contact. None were in the water in the glass dish; but upon lifting this, two were found in a little pool beneath it, a position which they had evidently reached by going down from the nest into the deeper and wetter layers of the loose debris until they reached the wet surface of the more solid layer at the bottom. In another little pool two others were found. These larvæ, like those in the loose soil above, were very active when disturbed. They placed themselves, as do the aquatic larvæ in their natural habitat, at the edge of the water with the head almost out and the back hardly covered. Eleven were found in all in different parts of the terrarium at this time. The others had gone down too far in the loose soil to be located without a more complete overturning of the terrarium than was deemed advisable. Five of the specimens were taken and preserved for later study (stages *B* and *C*). There is no means of knowing the exact date of hatching of each of these specimens, which may have been anywhere from one to five days old; it is very probable, however, that two of them, which were taken from the water (stage *C*) and which were somewhat larger than the others, were hatched earlier, especially as the others (stage *B*) were taken from near the nest.

During the next few days the terrarium was examined from time to time to locate the remaining larvæ and to discover any change in their activities. They never at any time showed the slightest instinct to go directly to the little pool of water which was now arranged for their reception but would often leave the nest in exactly the opposite direction. In general they made their way, as has been noted above, deeper and deeper into the loose soil until they finally reached the little areas of very shallow water on the surface of the hard soil at the bottom. Under natural conditions the burrows which penetrate the soil in which the *Desmognathus* lives must furnish natural and easy channels for the descent of the larvæ to a level at which the burrows would contain little pools of water. From here it is an easy matter for them to make their way beneath the loose layer of vegetable debris to the open water of the neighboring brook. Possibly an instinct of the larvæ to remain in contact with the body of the mother serves as an additional guide to them, as they would thus naturally follow her movements through the burrows. That the larvæ, like the adults, are thigmotropic, would also lead them to confine their movements to the crevices in the soil; a negative heliotropism may also assist in determining the downward direction of their movements.

On October 5, five more specimens were killed (stage *D*); of these four were found to have been more or less mutilated, having suffered the loss of a tail, or one of the limbs or both tail and limbs. Since it frequently happens that a considerable proportion of larvæ collected from their natural habitat show mutilations similar to these, it is evident that the cause in question is one which is operative under natural conditions. It is possible that the injuries are inflicted by the stronger larvæ upon their weaker brothers, especially as the injured individuals are usually below the average size. It is more probable, however, that the mother, under the pressure of a paucity of food, found her offspring tempting morsels. The fact that two of the brood were shown by the final count to be missing suggests that this conjecture is the correct one, especially as the larvæ themselves are very hardy and will live for weeks in the laboratory under very adverse conditions. I have noted in several instances similar total disappearance of larvæ from terraria in which they had been placed in company with one or two adults, and in two cases have found whole larvæ in the stomachs of adults which were in the terrarium from which larvæ had disappeared.

On October 6 one specimen more was killed (stage *E*), and on October 14 all of the remaining ones which could be found, three in number (stage *F*).

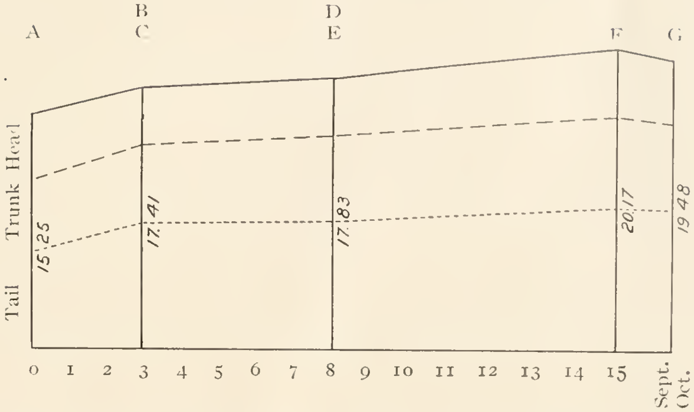
Since by this time the larvæ had not only reached the water in the bottom of the jar but had attained the size and proportions of the aquatic larvæ collected from their natural habitat at this season of the year (cf. Table I., and Graphs I. and II.), it may be considered that the duration of the terrestrial larval period is approximately 15 or 16 days, the average age of stage *F*. That the duration of the terrestrial period is very variable will, however, be inferred from the fact that in the case of this particular brood a difference in activity led some of the larvæ to the water several

TABLE I.

(The measurements given in this table are approximately those of the head, trunk, and tail, respectively, though, for convenience in measuring, the axilla and the anterior angle which the posterior limbs make with the body were taken as the somewhat arbitrary limits of these regions, cf. Plate II., 11, dotted lines.)

TERRESTRIAL LARVAL PERIOD.

Designation and Age.	Number of Examples.	Column I. Actual Sizes (Cf. Graph I.), Mm.				Column II. Proportionate Lengths of Body Regions (Cf. Graph II.)		
			Maximum.	Minimum.	Average.	Maximum.	Minimum.	Average.
Stage *A*. 0 days.	2	Head	4.00	4.00	4.00	.258	.267	.262
		Trunk	5.00	5.00	5.00	.323	.333	.328
		Tail	6.50	6.00	6.25	.419	.400	.410
		Total......	15.50	15.00	15.25	1.000	1.000	1.000
Stages *B* and *C*. Average age 3 days.	5	Head	4.00	4.00	4.00	.219	.242	.234
		Trunk	5.50	5.00	5.33	.301	.303	.312
		Tail	8.75	7.50	8.085	.479	.454	.454
		Total......	18.25	16.50	17.415	1.000	1.000	1.000
Stages *D* and *E*. Average age 8 days.	3	Head	4.25	4.00	4.17	.233	.229	.234
		Trunk	5.75	5.50	5.58	.315	.314	.313
		Tail	8.25	8.00	8.08	.452	.457	.453
		Total......	18.25	17.50	17.83	1.000	1.000	1.000
Stage *F*. Average age 15 days.	3	Head	4.50	4.50	4.50	.220--	.225	.223
		Trunk	6.25	6.25	6.17	.305–	.3125	.306
		Tail	9.75	9.25	9.50	.476–	.4625	.471
		Total......	20.50	20.00	20.17	1.000	1.000	1.000



GRAPH I., showing the growth in length during the terrestrial larval period (cf. first column of statistics, Table I.). Lines *A*, *BC*, *DE*, and *F* correspond to the similarly designated stages, the average age of each stage in days being indicated by the number at the bottom of the line and the average length (× 2) by the length of the line. Line *G* shows, for comparison with *F*, the average length of 42 aquatic larvæ collected during the months of September and October.

days in advance of others; and the differences in the distances of nests from the water would introduce still another variable factor.

While the limits of the terrestrial larval stage are somewhat indefinite, the structural changes which take place during this period are very important, and for the study of these changes the above described material may be tabulated as follows (see Plates I. and II.):

Stage A.—Two specimens killed September 25. Just hatched. Average length 15.25 mm.

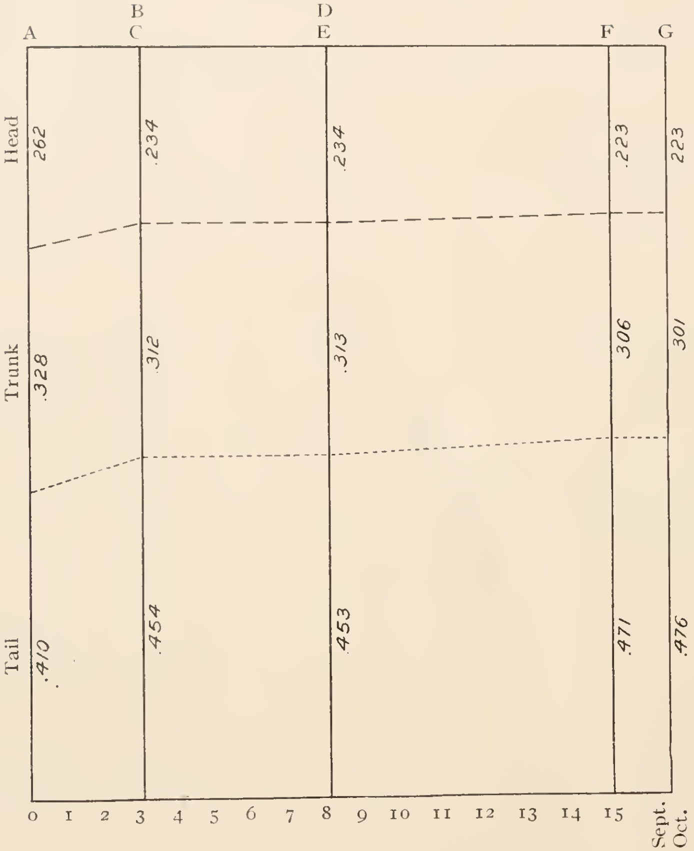
Stage B.—Three specimens killed October 1. Average age 3 days. As these were taken from near the nest the actual age of all of them is probably less. Average length 16.83 mm.

Stage C.—Two specimens killed October 1. Age as in the case of stage *B*, but as these two were taken from the pool of water at a greater distance from the nest, their actual age is probably above the average of 3 days. Average length 18 mm. Average length of *B* and *C* together is 17.415 mm.

Stage D. Five specimens killed October 5. Average age 7½ days. Average length of the two uninjured ones 17.652 mm.

Stage E.—One specimen killed October 6. Average age 8½ days. Length 18.25 mm. Average length of *D* and *E* together is 17.83 mm.

Stage F.—Three specimens killed October 14. Average age 15 days. Average length 20.17 mm.



GRAPH II., showing a comparison of the proportionate lengths of the body regions during the terrestrial larval period (cf. second column of statistics, Table I.). Lines *A*, *BC*, *DE*, and *F* refer to the various terrestrial stages as in Graph I. and line *G* shows, for comparison with *F*, the proportionate lengths of aquatic larvæ collected in September and October.

EXTERNAL FEATURES OF THE TERRESTRIAL LARVA.

The most conspicuous feature of the newly hatched larva (stage *A*, cf. Plate I., 1 and 2)[1] is the bulging form of the abdomen, due to the presence of a considerable mass of as yet unconsumed yolk material. The skin over this region is thin, unpigmented, and transparent, and the yellowish white appearance due to the yolk cells beneath it contrasts strongly with the deeply pigmented surface of the rest of the body. Along the back on each side of the mid-line the general gray pigmentation is interrupted by a row of rather poorly defined, rounded areas of lighter color, in some cases showing a slight brownish tinge. These spots are somewhat irregularly arranged, usually not exactly opposite each other on the two sides. The number on each side in the trunk region varies from 7 to 10, and the series extends nearly throughout the caudal region where it finally leaves off by a gradual diminution in the size of the areas.

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[1] Plates will be found at the end of the second part of this paper in the next issue of the BIOLOGICAL BULLETIN.

The tail is short and shows only a slight beginning of the dorsal and ventral folds which are later to form the tail fin. The gills are well developed and when the specimen is placed in water, they stand out conspicuously from the sides of the body. There are three of these gills upon each side. They are situated upon the lateral surface dorsal to the gill slits, in an oblique line, the most anterior one being more ventral in position and the most posterior one the more dorsal. This latter one is the longest and possesses from five to seven branches some of which bend conspicuously forward; the middle gill has usually five branches, and the ventral one three.

The legs, particularly the posterior ones, are stout and well developed, and are longer in proportion to the length of the trunk region than at any later period of development. Although the quick jerky motions with which the newly hatched larvæ, when disturbed, propel themselves through the loose soil seem to be performed with the whole body rather than with the legs, there are undoubtedly slower movements of the larvæ when left undisturbed, which involve the action of the legs. This greater proportionate size of the hind legs is, it will be remembered, one of the features distinguishing *Desmognathus* from *Spelerpes* larvæ, and is undoubtedly accounted for by the demands of this short terrestrial period preceding the aquatic larval life; since the eggs of *Spelerpes* develop, and the larvæ hatch, in the water, there is no need in this species for the early development of legs as organs of locomotion.

During the period of terrestrial life, important changes take place which result in a decided transformation in the appearance of the larvæ. The yolk protuberance rapidly diminishes, as will be seen by comparing Plate I., 1 to 9, so that by the time stage *D* is reached (11 days, at the latest, from the time of hatching), practically all external evidence of the presence of yolk has disappeared, though dissection and sectioning both demonstrate the presence of an abundance of yolk granules in the walls of the intestine. With this reduction of the yolk protuberance, the medial edges of the *rectus abdominalis* muscles, widely separated at the time of hatching, gradually approach each other and finally meet in the *linea alba*.

The changes in the shape and appearance of the tail are noteworthy. The tail becomes longer, so that from being at the time of hatching only 41 per cent. of the total body length, by the time the larvæ have reached the water and become typical aquatic larvæ (stage *F*, averaging only 15 or 16 days from the time of hatching) this same measurement is found to be over 47 per cent. of the total body length. With the increase in length of

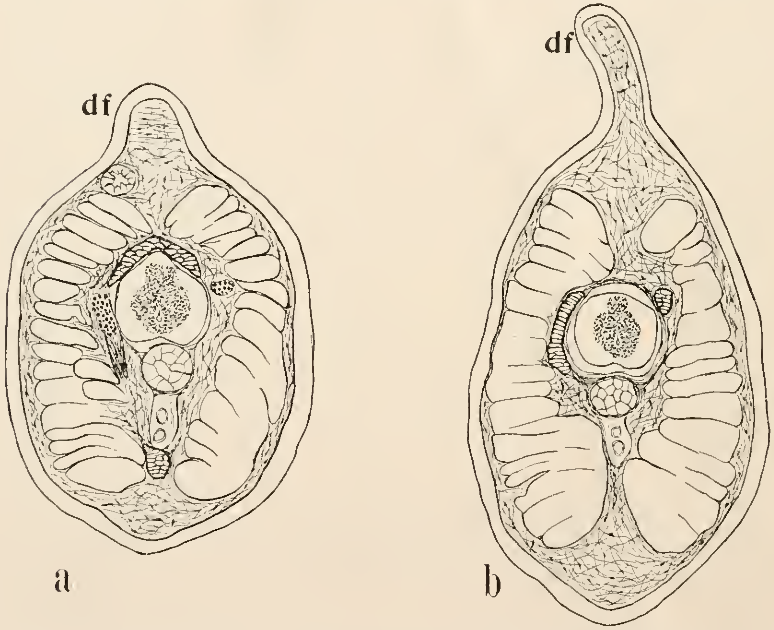

FIG. 7. Cross sections, at approximately the same level, of the tails of (*a*) a newly hatched larva, terrestrial stage *A*, and (*b*) an aquatic larva, showing the difference in proportions, and the development of the dorsal fin (*df*). For the level of the sections cf. Plate I., Figs. 1 and 10, Drawn with Abbé camera. × 60.

tail comes the change in its shape owing to the development of the median fin both on the dorsal and ventral side. Dorsally it begins at the level of the posterior margin of the pelvic girdle, where a slight depression in the mid-dorsal line may be seen when the animal is viewed from the lateral aspect. This dorsal portion of the fin, which finally in its widest part comes to contribute from one third to two fifths of the entire dorso-ventral dimension of the tail, extends to the extreme tip of the tail, where its continuity with the ventral portion of the fin is interrupted by the slender tip of the vertebral column and its associated muscles. The ventral portion of the fin is neither so wide nor so extensive as the dorsal, and it narrows anteriorly to end at a point somewhat posterior to the cloaca. The increase in length of the tail is the principal factor in the change in length proportions of the body shown in the second column of Table I., and in Graph II. Thus while, during the terrestrial period, the head actually increases only about 12 per cent. in length, and the trunk 23 per cent., the tail increases 52 per cent. (cf. Table I., 1st column). These changes in the tail are obviously a preparation for the aquatic life which is so soon to follow.

Not only do the proportionate lengths of the regions of the body thus alter, but the actual growth is, during the first few days after hatching, very rapid, as will be seen from the 1st column of Table I., and also by comparison of Plate I., 1–9, all drawn to the same scale. Thus at the end of 16 days the total increase in length (20.17 – 15.25 = 4.92 mm.) is 32 per cent. of the length of the body at the time of hatching.

The Alimentary Canal of the Terrestrial Larva.

Internally no structure exhibits more striking and fundamental changes during the terrestrial period than the alimentary canal. In the walls of the alimentary canal at the time of hatching there is still a considerable supply of yolk material, although several days previous to hatching (*i. e.*, in the embryo of 30 days' development, 13 mm. long), the yolk material contained in the cells of the other tissues has been already quite consumed. In the newly hatched larva, indeed, the digestive tract has so far progressed in its development that œsophagus and stomach have already completed their histogenesis, and the duodenum and rectum practically so; leaving the yolk material in the remaining intestinal region.

The lining of the œsophagus is thrown into longitudinal folds, and is composed of columnar cells of the ciliated type with a large number of active unicellular glands interspersed among them (Fig. 8, *b*). The stomach is sharply differentiated from the œsophagus on the one hand and from the duodenum on the other. It is divided into cardiac and pyloric regions, the former expanded, piriform, and glandular, the latter tubular and far more muscular. In both these regions of the stomach the unicellular type of gland is lacking, but there are numerous multicellular glands of the convoluted tubular type, opening

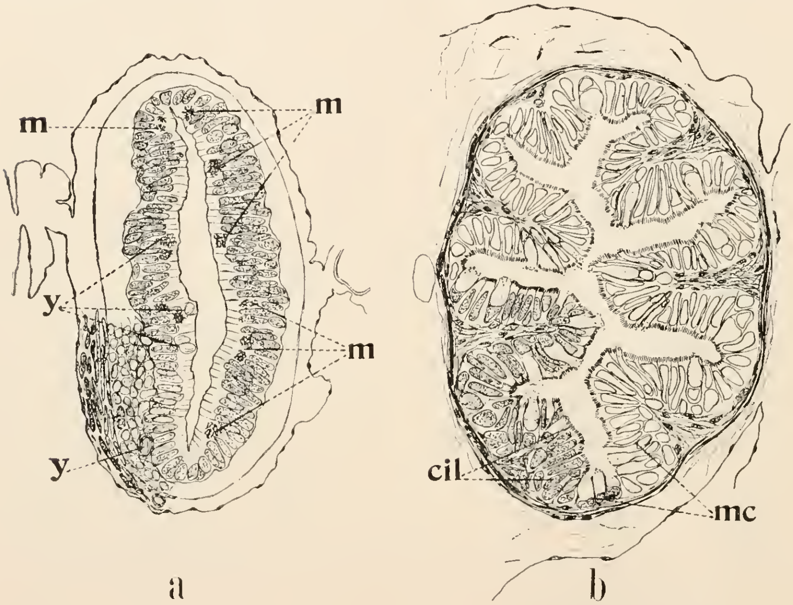

FIG. 8. Cross sections of the œsophagus of (*a*) a 13 mm. embryo, and (*b*) a newly hatched larva, showing the change in the character of the epithelial lining which takes place during the few days previous to hatching. (*a*) *m*, mitosis; *y*, last traces of yolk granules in the epithelial cells; (*b*) *cil*, ciliated epithelial cells; *mc*, mucous cells. Note the absence of folds in (*a*), and of mitosis in (*b*). Drawn with Abbé camera. × 103.

between the many irregular folds of the lining mucous membrane. The cells of the mucous membrane are sub-columnar in form, with a smooth exposed surface. The final stages of this histogenesis must take place with great rapidity, during the last few days before hatching. In the 13 mm. embryo neither folds, ciliated cells, nor glands of any kind have appeared in the alimentary canal; the stomach is differentiated from the œsophagus, only in being less flattened dorso-ventrally, and containing in its cells a larger amount of yolk; there is an enormous amount of mitosis in progress especially in the œsophageal

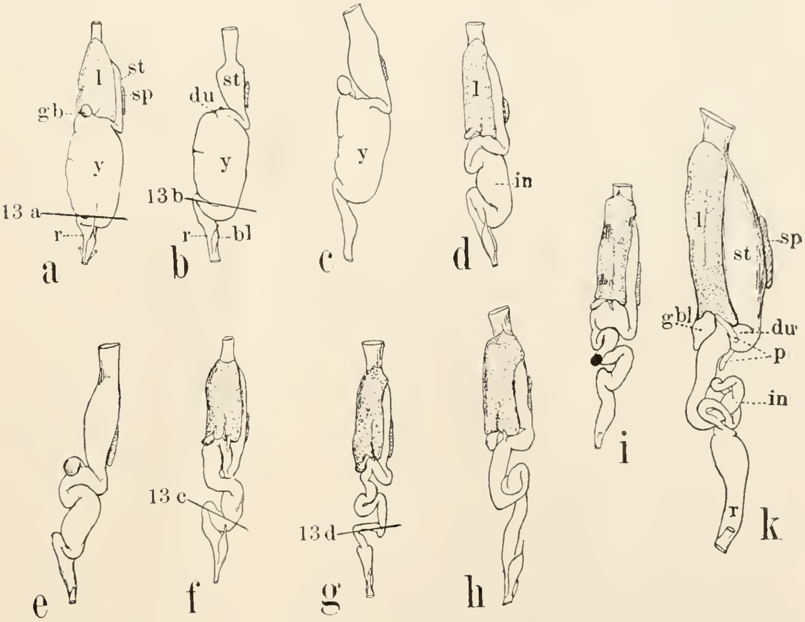

FIG. 9. A series of dissections of the alimentary canals of various stages of *Desmognathus fusca*, showing the gradual consumption of the yolk and the differentiation of the intestine. (*a*) terrestrial larval stage *B*, 3 days old; (*b*) same stage as (*a*) with the liver removed; (*c*) terrestrial larval stage *D*, 7½ days old; (*d*) terrestrial larval stage *F*, 15½ days old; (*e*), (*f*), (*g*), (*h*), aquatic larval stages collected in September and October; (*i*) aquatic larval stage collected in May; (*k*) adult stage, length 45 mm.

Lines 13*a*, 13*b*, 13*c*, and 13*d* show the levels and directions of the respective sections of Fig. 13; *bl*, bladder; *du*, duodenum; *gbl*, gall bladder; *in*, intestine; *l*, liver; *p*, pancreas; *st*, stomach; *sp*, spleen; *r*, rectum; *y*, yolk mass. Drawn with Abbé camera. × 4.

region (Fig. 8, *a*), undoubtedly the process of cell proliferation which results in the formation of the longitudinal folds.

With equally rapid strides does the histogenesis of the intestinal region occur during the brief terrestrial period of larval life, until from the large yolk mass which fills and distends the body cavity of the newly hatched larva and furnishes nutriment for the rapidly growing animal, there is developed the entire length of intestine with its convoluted windings, ready for the digestion and absorption of ingested food material.

The changes in the external form of the intestinal region involved in this process may be demonstrated by comparison of the dissections shown in Fig. 9, from which it will be seen that there is gradually moulded out of the intestinal yolk mass a loop forming an *S*-shaped figure which consists of an anterior portion lying upon the right side, into which the duodenum opens, and a posterior portion lying upon the left side, from which the rectum leads. Both of these regions, particularly the left and more posterior loop, are for a considerable time enlarged through the presence of the remaining yolk material as indicated by the condition shown in stage *F*, (Fig. 9, *d*) the oldest of the terrestrial larvæ. Gradually, however, concomitant with the consumption of the last remnants of yolk material and the consequent diminution in the diameter of the intestine, there occurs a still further increase in its length, so that each of the two main loops acquires two or three subsidiary ones variable, of course, in relative position with constantly varying peristaltic conditions (cf. Fig. 9, *e*, *f*, *g*, and *h*).

The last part of this process of differentiation of the intestine is apparently not usually accomplished until after the larva has reached the water, since frequently in the early fall aquatic larvæ are found with the intestine in the condition shown in *d*, *e* and *f*, Fig. 9, exactly similar to the condition found in my oldest terrestrial stage *F*. Food may be ingested many days previous to the final disappearance of the yolk; in fact, the stomach of one specimen of stage *C* (terrestrial larva, average age 3 days and greatest possible age 5 days) contained a small copepod, while there was another in the intestine; and the specimen of stage *D*, from which the dissection shown in Fig. 9, *C*, was made, had 30 copepods in the stomach, and in the stomach of another specimen of the same stage 6 copepods were found.[1] In all the cases which I have examined, of aquatic larvæ in which yolk was still present, feeding has been extensive and fragments of partly digested aquatic animals, in the main copepods and insect larvæ, have been found not only in the stomach but in various regions of the intestinal tract. Whether the early assumption of its digestive function on the part of the alimentary canal has the effect either to retard the final consumption of yolk material through the supply of nutriment from external sources, or to hasten its consumption through the stimulation of the histogenesis of the walls of the canal through use, I am at present unable to say. At any rate the excess of the supply of yolk material over the amount which the larva usually consumes before reaching the water with its ample supply of appropriate food, is undoubtedly an adaptation to a possible prolongation of the terrestrial period such as might easily occur if the eggs happened to be laid at a greater distance than usual from the water, or if an unusually dry season during the period of development of the egg should diminish the water supply in the brook and in the burrows leading to it and thus lengthen the period which might elapse before the larvæ should reach the water.

Internally the differentiation of the intestine from the mass of yolk cells is a somewhat complicated process, the detailed account of which must be reserved for a more technical treatment of the whole subject of the histogenesis of the alimentary canal. Certain salient features of the process may, however, be discussed here.

At the time of hatching the duodenum is in what may be termed the transition stage in its development (*d*, Fig. 12). Anterior to the point where the bile duct leads into the duodenum from the fully formed liver, yolk granules have disappeared, as they have also from the cells of the liver and pancreas and their ducts. Posterior to the opening of the bile duct into the duodenum, there is for some distance a well-defined lumen, but the epithelial cells lining it contain a considerable number of yolk granules, although among these cells there have already appeared well-developed mucous cells which are filled with their secretion and are already discharging it into the lumen. The duodenum leads rather abruptly into the right anterior region of the cylindrical yolk mass, beneath the posterior margin of the liver.

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[1] All of these individuals, though ranking as terrestrial larvæ, happen to be among the more precocious ones which had reached the water, where they had access to food.

In this mass of yolk cells two types of cell may be distinguished, a smaller and a larger (*ys* and *yl*, Fig. 11). The smaller ones lie peripherally, have a spherical or slightly columnar form with a well-defined nucleus usually in the peripheral end of the cell.

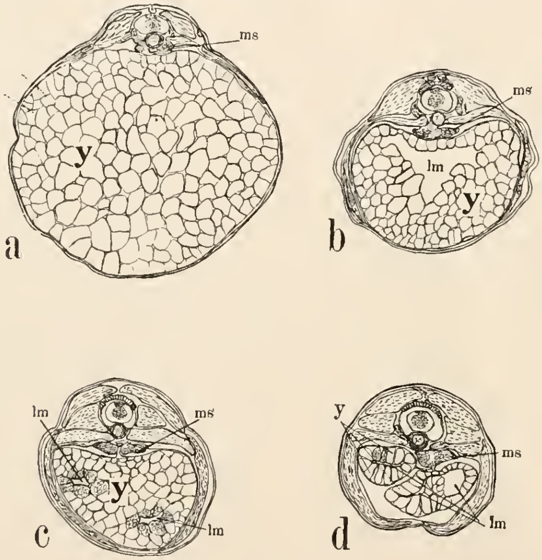


Fig. 10. Cross sections at the same level through the middle of the body of (*a*) a 13 mm. embryo; (*b*) newly hatched larva, terrestrial stage *A* (cf. Fig. 12, *a*. and Plate I., Figs. 1 and 2); (*c*) larva 3 days old, terrestrial stage *C* (cf. Fig. 12, *c*, and Plate I., Figs. 5 and 6); (*d*) larva 15½ days old, terrestrial stage *F* (cf. Fig. 12, *d*, and Plate I., Fig. 9). Note the gradual reduction of the mass of yolk cells (*y*) and the formation of the intestinal lumen (*lm*); *ms*, mesonephros. Enclosure between the dotted lines in (*a*) indicates the location of Fig. 11. Drawn with Abbé camera. × 12½.

The cell walls are definite, the contained yolk granules of varying size, but among them relatively few large ones. The remaining portion of the yolk mass consists of enormous cells which are often multinucleate, with very large, irregular nuclei. The cell walls are not always clearly defined and in many regions seem to rupture easily. One must, however, remember the great liability to imperfect preservation of the middle region of the yolk, which might account for apparent imperfection of cell walls. Although during the earlier part of development within the egg, these yolk cells of the large type contain both large and small yolk granules with, however, a preponderance of the large ones, by the time the larva hatches practically all of the smaller granules seem to have been consumed, and as a result the large granules alone appear, and these not very closely crowded together. It is from the cells of the smaller type, the peripheral ones, that the future epithelial lining of the intestine will arise, while the large cells which form the bulk of the yolk mass may be regarded as purely nutritive in function, since from these apparently no tissue is derived. If this distinction goes back, as seems probable, to the early differentiation of the cells of the blastula into the peripheral ones containing small yolk granules, and the central mass of cells containing large yolk

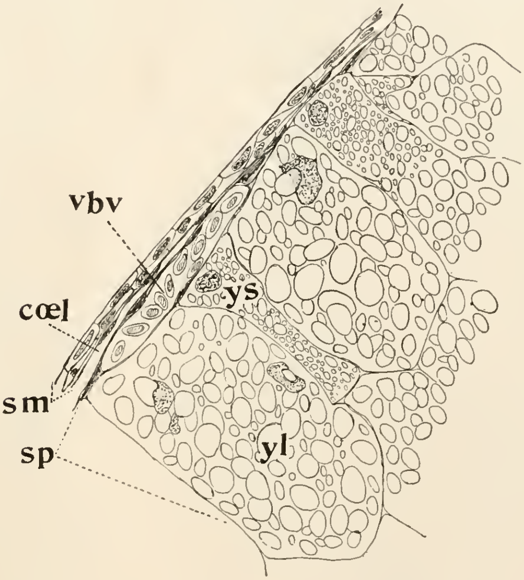


FIG. 11. Detail of region indicated between the dotted lines in Fig. 10 (*a*). *Coel*, coelomic cavity; *sm*, somatopleure; *sp*, splanchnopleure; *vbv*, vitelline blood vessel; *yl*, yolk cells of the larger (nutritive) type; *ys*, yolk cells of the smaller type. Abbé camera. × 273.

granules (Hilton, '09), we see again the approach to an intermediate position of the *Desmognathus* egg between the holoblastic and meroblastic types, this mass of large cells of a purely nutritive character being comparable to the undivided yolk mass of the meroblastic egg. The tardy segmentation of this mass of yolk material, which Hilton pointed out, and the later breaking down of the cell walls of these large nutritive cells, which seems to appear in some cases, corroborate this view.

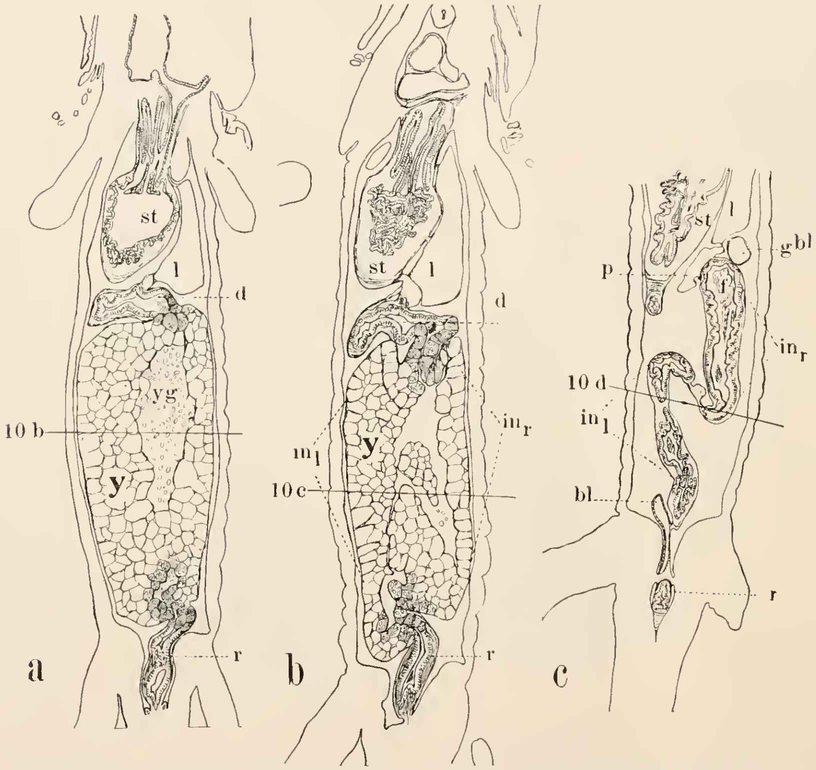


FIG. 12. Horizontal sections (viewed dorsally) of (*a*) terrestrial larva at time of hatching (stage *A*, cf. Plate I., Figs. 1 and 2), (*b*) terrestrial larva 3 days old (stage *C*, cf. Plate I., Figs. 5 and 6), (*c*) terrestrial larva 15½ days old (stage *F*, cf. Plate I., Fig. 9). Lines 10*b*, 10*c*, and 10*d* indicate respectively the levels of the cross sections shown in Fig. 10. *Bl*, bladder; *d*, duodenum; *gbl*, gall bladder; in_l, left loop of intestine; in_r, right loop of intestine; *l*, liver; *p*, pancreas; *st*, stomach; *r*, rectum; *y*, yolk mass; *yg*, yolk granules in the lumen of the yolk mass. Drawn with Abbé camera. × 12½.

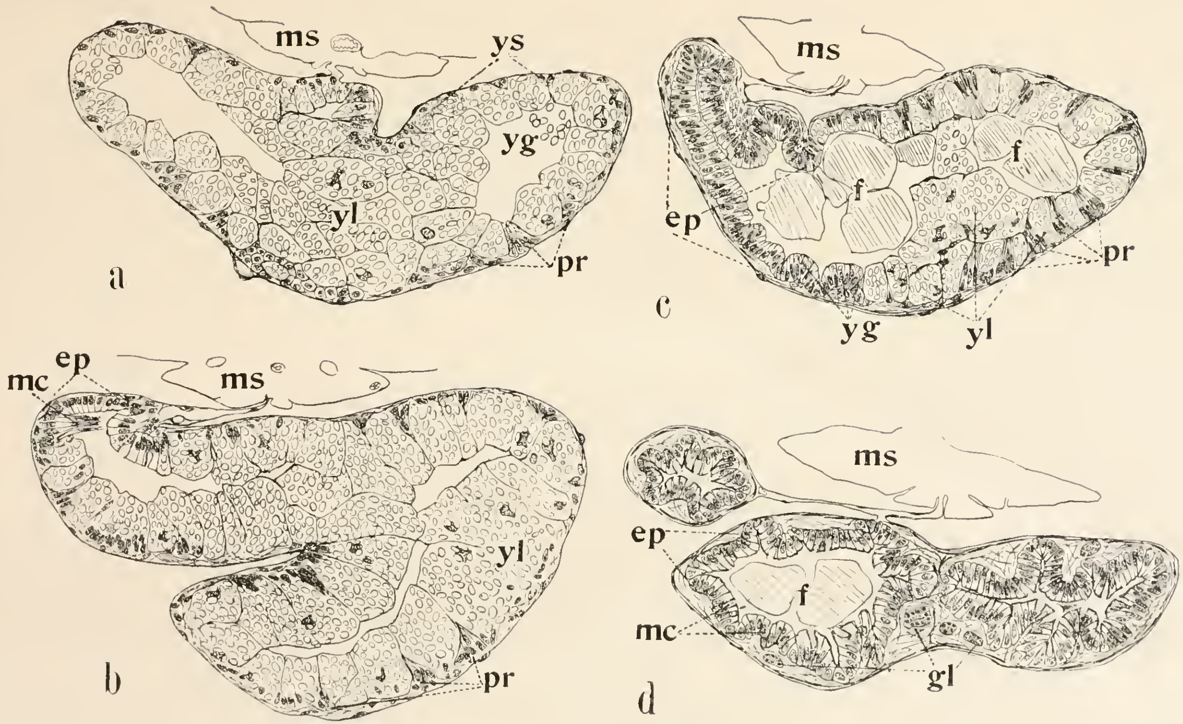


FIG. 13. Sections showing four stages on the differentiation of the posterior part of the intestine from the mass of yolk cells, from cross sections through approximately the same region of the body; (*a*) terrestrial larva at the time of hatching,(*b*) terrestrial larva 3 days old, (*c*) and (*d*) two aquatic larvæ collected in September. The levels and the directions of the sections with respect to the yolk mass and intestine are indicated approximately by lines 13*a*, 13*b*, 13*c*, 13*d*, respectively, in Fig. 9 (*a*), (*b*), (*f*) and (*g*). *Ep*, regions of functional epithelium; *f*, food in the intestinal tract; *gl*, multicellular glands of the intestinal mucous membrane; *mc*, mucous cells; *ms*, mesonephros; *pr*, regions of proliferation of epithelial cells to form future intestinal folds; *yg*, yolk granules; *yl* and *ys*, yolk cells of the large (nutritive) and small (tissue-forming) types respectively. Drawn with Abb écamera. × 60.

Although there is at the time of hatching a considerable cavity within the yolk mass (Figs. 10, *b*, and 12, *a*), its walls are irregular and poorly defined. The anterior region of the cavity is located more upon the right side and communicates with the lumen of the duodenum, while more posteriorly the cavity swings somewhat to the left side, where it finally communicates with a partly differentiated posterior region of the intestine which extends transversely from left to right to lead into the rectum. Thus the *S*-form so soon to appear in the external moulding of the intestine (Fig. 12, *c*) is already indicated in the lumen of the yolk mass.

The further differentiation of the intestine from the mass of yolk cells (Fig. 13) involves a double process consisting of (1) rapid reduction and disappearance of the yolk material contained in the nutritive cells (*yl*), which may from time to time be sloughed off into the lumen, or may be ruptured, thus discharging their contents into the lumen; and (2) the proliferation of the peripheral cells (*pr*), the planes of division being radial with relation to the lumen of the intestine, and resulting in the formation of the anlagen of the future folds of the mucous membrane, in the form of groups of tall columnar cells which, elongating, push their way to the lumen between the remaining nutritive cells (Fig. 13, *c*). These nutritive cells thus come to lie in the furrows between the folds, from which place they gradually disappear. Mucous cells develop early from certain of the peripheral cells, and are of a tall columnar type, corresponding to the form of the other epithelial cells among which they lie. Multicellular glands (*gl*) develop later from a proliferation of cells at the bases of the folds.

Although this differentiation of the intestine progresses almost simultaneously throughout the main part of the yolk mass, it proceeds a little more rapidly at both anterior and posterior ends, than in the middle of the yolk region. Thus at the time of hatching the anterior part of the duodenum and practically all of the rectum are fully differentiated, and the regions of intestine immediately adjoining these are in that stage of development in which the anlagen of the intestinal folds are already formed, a condition which is not reached by the larger portion of the intestine until several days later.

The Skin of the Terrestrial Larva.

The epidermis of the newly hatched larva (Plate IV., 19, 20, 21, and 22) is in the typical larval amphibian condition in that it consists of two layers of cells; these are for the most part subspherical in shape with polygonal outlines when viewed in a section parallel with the external surface. They possess large nuclei which in both layers show a small precentage of mitosis. The cells of the outer layer have a deeply staining cuticular border on the exposed surface, forming all together a continuous cuticular layer (*cu*) covering the external surface. In general over the dorsal and dorso-lateral surfaces the outer layer of the epidermis is the thinner one and its cells are slightly flattened. Over the lateral and ventral surfaces the outer layer is the thicker, the cells here approaching the columnar form, while their nuclei are piriform with the pointed end directed toward the outer surface and often nearly reaching it; in these regions the inner layer is considerably thinner and the nuclei smaller. Over the region which is still distended with yolk, both layers are thin, the cells of the inner one approaching the squamous type. In a few limited regions as, for example, the lateral wall of the body beneath the gills, the whole epidermis is very thin, and the cells of both layers decidedly of the squamous type; as seen in cross section their nuclei alternate, and the whole appearance may thus even simulate a single cell layer.

Beneath the deeper layer of epidermal cells in practically all regions of the body except the snout, there is a dense corium which fits closely to the deeper layer of cells, thus following their contour. This corium is a little thicker than the cuticular layer of the outer cells and is from one eighth to one sixth as thick as the whole epidermis.

In those regions of the body which are pigmented, the dorsal and lateral surfaces, the pigmentation is threefold. The external layer of epidermal cells possesses an intracellular pigmentation in the form of a layer of pigment granules (*pgg*) immediately beneath the cuticular layer and forming a sort of cap over the nucleus. In addition to this intracellular pigmentation there may be seen, between the cells, delicate intercellular ramifying branches (*pgbr*) of pigment cells (*pgc*) of the connective tissue type, the body of the cell often lying between the two layers of epidermal cells. These branches seem particularly to embrace the cells of the external layer, though occurring between the cells of the deeper layer also. Finally, beneath the corium and closely applied to it, is an interrupted layer of enormous patches of pigment matter which, however, does not seem to penetrate the corium and encroach upon the epidermis. In the unpigmented ventral region all of these forms of pigmentation are lacking.

In comparison with the skin of the 13 mm. embryo the skin of the newly hatched larva of 15.5 mm. impresses one as a fully formed structure (Plate IV., 17 and 18). In many regions of the 13 mm. embryo the epidermis is very thin, and although two layers of cells are present, the nuclei, particularly of the outer layer, are often so widely separated, owing to the flattened form of the cells, as to seem in a vertical section through the skin to be entirely lacking for considerable distances (cf. Fig. 11). There is only the merest trace of cuticular structure in the external border of the outer cells, and practically no corium beneath the lower cells. Branched pigment cells occur both beneath and between the epidermal layers but there is no evidence whatever of intracellular pigmentation of the epidermis. The embryonal character of the epidermis is evidenced by the slightly rounded external contour of the outer layer of cells, and by the large size of the nuclei, which, moreover, present an enormous amount of mitosis (*mt*) in both layers. As might be expected from the fact that the larval two-layered condition has already been reached, the mitotic figures are oriented with their axes parallel with the external surface of the body, and the process is, of course, giving rise to an increase in the number of cells in each layer. This condition of general mitosis contrasts sharply with the limited amount of mitosis in progress in the epidermis of the newly hatched larva, where the figures are, moreover, in late anaphase or telophase.

There occur after hatching but few changes in the skin of the larva to complete this rapid transformation from the embryonal condition to that which is to serve the animal during its eight or more months of larval life (Plate IV., 23, 24, and 25). These changes, which take place during the brief terrestrial period, involve no mitosis, not even in the rapidly growing tail and tail fin. They consist, rather, in a readjustment and rearrangement of the cells to suit the changes in the shape and proportions of the body, the full quota of cells having been already formed in the mitotic stage preceding hatching. Thus over the growing tail fin the epidermal cells become stretched apart, the cells of the deeper layer alternating with those of the external layer and in some cases actually reaching the external surface. In the ventral region of the body where the area of the surface is rapidly diminishing with the reduction of the yolk, the cells lose their flattened form and become subspherical or even columnar.

Over all parts of the body with limited exceptions, there is a slight increase in the thickness of the skin from various causes. The cuticular layer of the external cells increases slightly in thickness, probably, however, at the expense of the rest of the cell; the dense corium underlying the epidermis becomes a little thicker; the chief increase comes, however, from that change in the inner layer of epidermal cells which is the most conspicuous change in the skin during the terrestrial larval life, the distension of the cells as if from a condition of turgor. This distended condition soon becomes more marked in certain cells than in others, and these eventually form the greatly enlarged, vacuolated cells of the type described by Leydig ('53) and subsequently designated the "Leydig cells" of the epidermis (*lc*). These show a somewhat reduced nucleus and a large cytoplasmic region in which a loose reticulum sometimes appears, but which with most stains gives the effect of a clear area between the nucleus and the thin cell membrane. This enlargement of certain cells naturally increases the thickness of the deeper epidermal layer. The Leydig cells press inward upon the corium and outward upon the cells of the external layer; they seem even to push around and between these latter cells but never to actually reach the external surface. The remaining cells of the deeper layer, which lie between the Leydig cells, become crowded together into a columnar form and reach in most cases from the corium to the outer layer of cells. The differentiation of the Leydig cells has already begun at the time of hatching and is most conspicuous

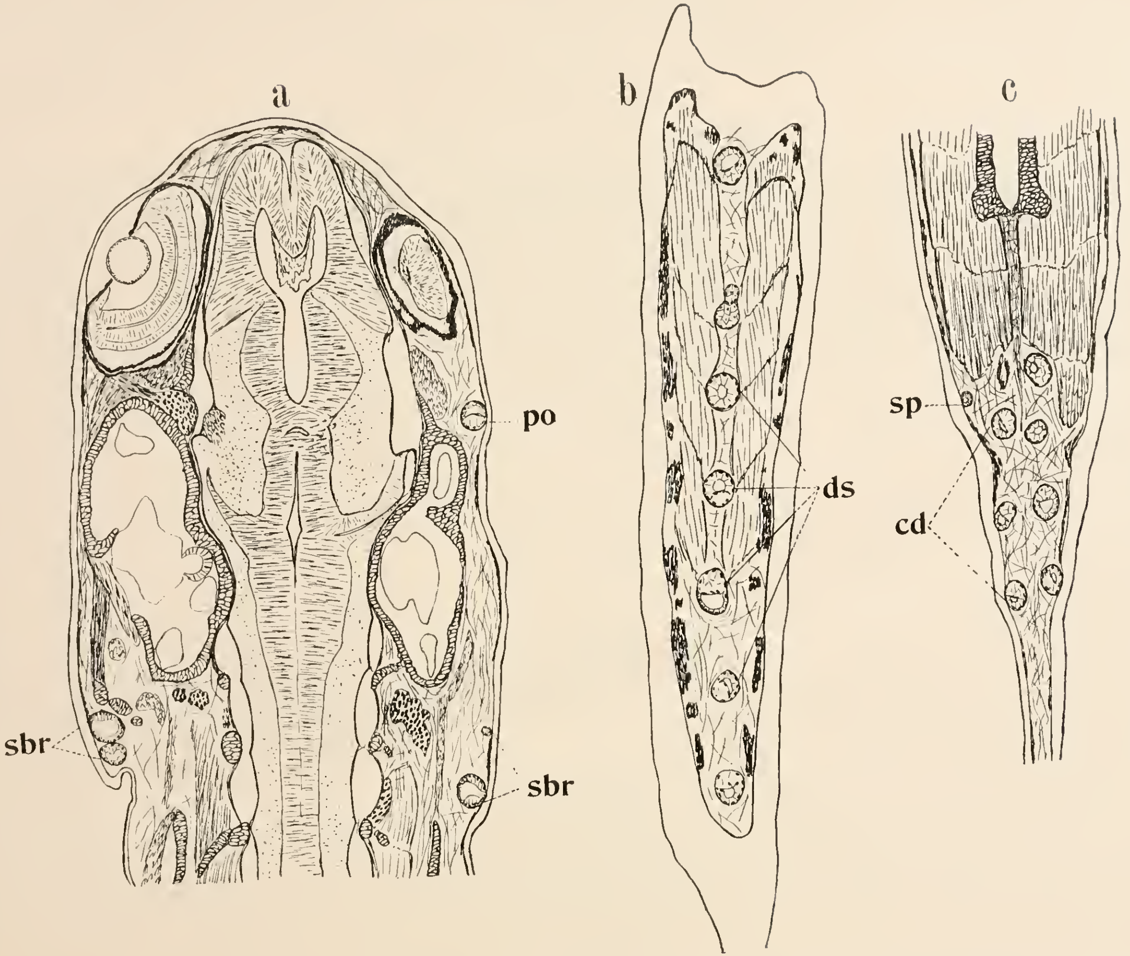


FIG. 14. Horizontal sections of *Desmognathus fusca* larvæ showing the distribution of larval acinous glands; (*a*) through the post-orbital (*po*) and the suprabranchial (*sbr*) groups of glands; (*b*) through seven of the mid-dorsal (*ds*) group; (*c*) through a portion of the double row of the tail (*cd*) at the level of the base of the dorsal fin; also showing a single sporadic gland (*sp*). Drawn with Abbé camera. × 32.5.

along the dorso-lateral regions; it proceeds so rapidly during the few succeeding days that in stage *D* the process is almost completed.

Two sets of highly specialized integumental organs have already made their appearance at the time of hatching. One of these consists of the neuromasts, or sense organs (*sn*) of the lateral line type, which are abundantly distributed over the head, especially the snout, and in two lines which extend along each side of the trunk, while a single line upon each side extends along the tail. These organs, each of which consists of a group of specialized epithelial cells, begin their development long before hatching and at the time of hatching appear to be fully formed so far as the number of cells entering into them is concerned. The surrounding accessory cells probably undergo some further modification.

The other organs to be mentioned are multicellular glands of the alveolar type. They are limited in number and occur in definite locations; a small group of two or three posterior to each eye (the post-orbital group, *po*, Fig. 14, *a*), a group of from one to three over the otic region (the supra-otic group), a larger group of from six to eight on each side of the head posterior to the otic region and dorsal to the gill bushes (the supra-branchial group, *sbr*, Fig. 14, *a*), and a single row approximately segmentally arranged along the middorsal line of the trunk (*ds*, Fig. 14, *b*), becoming, in the transition to the caudal region, a double row in slightly alternating, segmental pairs (*cd*, Fig. 14, *c*) lying along the line of junction of the dorsal finfold and the tail. With the exception of those at the posterior end of the caudal series and a few of the supra-branchial group, these glands are very large, measuring in diameter from two to eight times the thickness of the epidermis; and although they have developed from the epidermis they lie quite beneath both the epidermis and corium within the loose layer of subcutaneous tissue, and open to the exterior by a slender duct which leads through the skin (Fig. 15, *a*). Some of them have already made their appearance in the 18-day embryo as a mass of rapidly dividing epithelial cells, and in the 13 mm. embryo of 30 days' development are already discharging a granular secretion upon the surface of the skin. Thus the larva hatches fully equipped with a set of actively secreting glands which are to continue their activity throughout the larval life.

(*Concluded in the next issue.*)

Vol. XXIV. *April, 1913.* *No. 5*

BIOLOGICAL BULLETIN

THE LIFE HISTORY OF DESMOGNATHUS FUSCA.

(*Continued from March Issue.*)

INEZ WHIPPLE WILDER.

The Aquatic Larval Period.

Following the brief but very important terrestrial larval period, the aquatic larval period begins. As may be computed from the limits of dates during which egg-laying occurs (June 1 to Sept. 1), the probable duration of incubation (5 weeks), and the somewhat variable period of terrestrial life (1 to 2 weeks), the date of the beginning of the aquatic period can hardly be earlier than the middle of July or later than the middle of October. I have personally never known of aquatic larvæ being found between June 17 and September 1. By September 15 they are present in the brooks in great abundance, the majority of them with yolk still present in the intestinal walls, a condition not unlike that shown by stage *F* of the terrestrial larvæ and never as yet found by me in specimens collected in October. These facts point to early September as the time when the majority of the larvæ reach the water and begin their aquatic existence. The wide range of variation in the size and proportions of larvæ during September and October (cf. Table II. and Graphs III. and IV.) represents the inevitable difference in age resulting from the long period of egg laying.

As to the duration of the larval period, we have the statement of Reed and Wright ('09): "The larvæ transform from September to December, when they are from 18 to 20 mm. long." My own observation does not confirm this statement. It is a very significant fact that after the larvæ once appear in the water in September, they are found about equally abundantly during the fall, winter, and early spring months, and that, as shown by Table II. and its accompanying Graphs III. and IV., the average proportions and size of the body remain practically the same until late spring, when there occurs a marked increase in average size and a decided change in the proportionate lengths of the regions of the body in that the tail lengthens more rapidly than either the head or trunk. Structurally, also, the specimens taken during the fall and winter display none of the indications of approaching metamorphosis which are so unmistakable when they do appear in the May specimens. Moreover I have never found larvæ actually undergoing metamorphosis except in June, nor any of the very small recently metamorphosed adults except in June and the early part of July. The aquatic larval period would thus appear to extend practically through autumn, winter,

Table II.

Aquatic Larval Period.

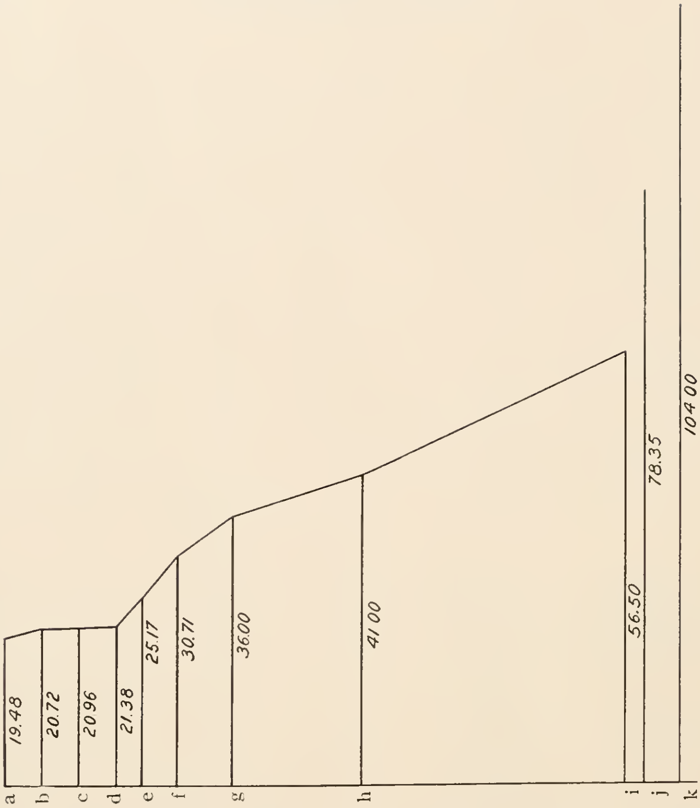
When Collected.	Number of Examples.	Column I. Actual Sizes (Cf. Graph III.), Mm.			Column II. Proportionate Lengths of Body Regions (Cf. Graph IV.).		
		Maximum.	Minimum.	Average.	Maximum.	Minimum.	Average.
September and October.	42	Head 5.00	4.00	4.34	.208	.261	.223
		Trunk 7.25	4.30	5.86	.302	.281	.301
		Tail 11.75	7.00	9.28	.489	.458	.476
		Total......24.00	15.30	19.48	1.000	1.000	1.000
November and December.	15	Head 5.40	4.00	4.56	.212	.234	.220
		Trunk 7.60	5.50	6.38	.298	.324	.307
		Tail 12.50	7.50	9.62	.490	.441	.473
		Total......25.50	17.00	20.72	1.000	1.000	1.000
January and February.	5	Head 4.55	4.25	4.51	.207	.224	.215
		Trunk 6.83	5.75	6.45	.311	.303	.308
		Tail 10.62	9.00	10.00	.483	.474	.478
		Total......22.00	19.00	20.96	1.000	1.000	1.000
March and April.	8	Head 4.50	4.10	4.395	.191	.217	.206
		Trunk 7.50	5.20	6.655	.319	.298	.311
		Tail 11.50	8.80	10.335	.489	.485	.483
		Total......23.50	18.10	21.385	1.000	1.000	1.000
May.	5	Head 6.00	4.75	5.12	.182	.218	.203
		Trunk 10.00	6.75	7.55	.303	.310	.300
		Tail 17.00	10.25	12.50	.515	.471	.497
		Total......33.00	21.75	25.17	1.000	1.000	1.000

ADULT PERIOD.

When Collected.	Number of Examples.	Column I. Actual Sizes (Cf. Graph III.), Mm.			Column II. Proportionate Lengths of Body Regions (Cf. Graph IV.).		
		Maximum.	Minimum.	Average.	Maximum.	Minimum.	Average.
Metamorphic stage. Collected June 17 to July 1.	3	Head 5.60	5.50	5.53	.193	.198	.195
		Trunk 9.40	7.75	8.72	.324	.279	.306
		Tail 14.00	14.50	14.17	.493	.522	.501
		Total......29.00	27.75	28.42	1.000	1.000	1.000
Small adults. Collected June 5.	2	Head 6.70	6.50	6.60	.191	.195	.193
		Trunk 10.50	10.30	10.40	.300	.309	.305
		Tail 17.80	16.50	17.15	.509	.496	.502
		Total......35.00	33.30	34.15 Average of metamorphic and smallest adults 30.71	1.000	1.000	1.000
Small adult. Collected Sept. 17.	1	Head..........	......	6.50	......	......	.181 –
		Trunk.........	......	10.50	......	......	.292 –
		Tail...........	......	19.00	......	......	.528 –
		Total..........	......	36.00	......	......	1.000
Small adult. Collected May 3.	1	Head..........	......	7.00	......	......	.171
		Trunk.........	......	12.50	......	......	.305
		Tail...........	......	21.50	......	......	.524
		Total..........	......	41.00	......	......	1.000
Miscellaneous set of adults. Collected in June and July.	10	Head 16.00	9.00	12.35	.154	.159	.158
		Trunk 31.00	16.50	23.65	.298	.292	.302
		Tail 57.00	31.00	42.35	.548	.549	.540
		Total.....104.00	56.50	78.35	1.000	1.000	1.000

It will be noted that in the above table the number of examples collected during September and October is greatly in excess of the number collected during the other months of the aquatic larval period. This fact is not due, as would seem to be indicated, to a greater abundance of the material during these months, but rather to the fact that these happen to be the months when most of my collection of *Desmognathus* material for laboratory purposes is made.

and spring, and to cover a somewhat variable period of from 8 to 10 months. The perennially running brooks in which they live are of course spring fed and do not freeze solid even though they are very shallow. In fact the brook which I have particularly studied as a typical *Desmognathus* habitat is said to never



Graph III., showing the growth in length during the aquatic larval period (lines *a–f*), metamorphosis (line *f*), and the first two years of adult life (lines *f–i*); also, for comparison with these, the average length (line *j*) and the maximum length (line *k*) of ten miscellaneous adults. Based upon the statistics given in the first column of Table II.

The time ratios are indicated by measurements horizontally. The vertical lines show, natural size, the average lengths of specimens as follows:

Aquatic larval period:

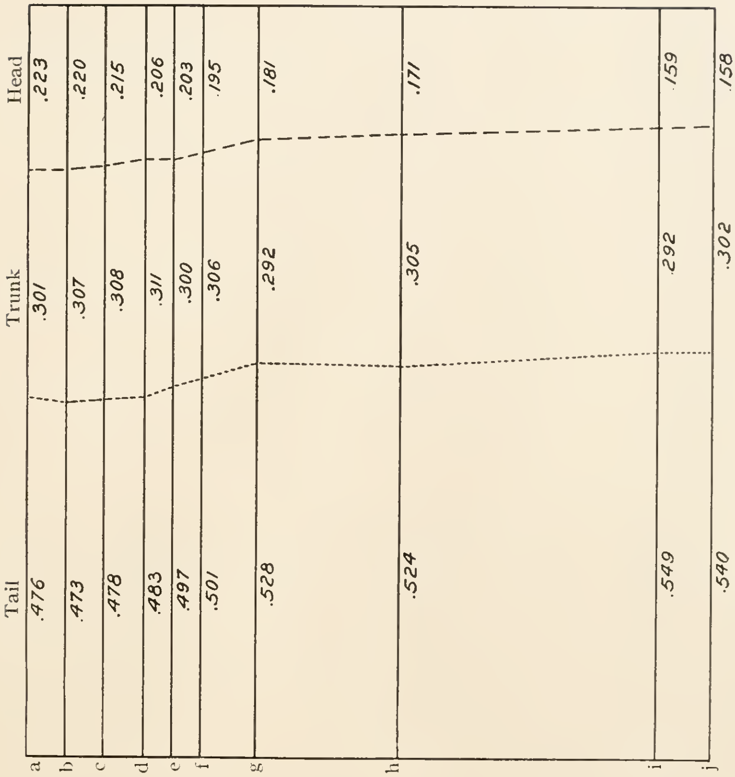
a, collected in September and October.
b, collected in November and December.
c, collected in January and February.
d, collected in March and April.
e, collected in May.

Metamorphosis:

f, collected in June and July.

Adult period:

g, collected in the September following metamorphosis.
h, collected in May nearly one year after metamorphosis.
i, collected in June and July two years after metamorphosis.
j, average of 10 miscellaneous adults.
k, maximum of 10 miscellaneous adults.



GRAPH IV., showing a comparison of the proportionate lengths of body regions during the aquatic larval period (lines *a–f*), metamorphosis (line *f*), and the first two years of adult life (lines *f–i*); also the proportionate lengths of body regions of the average of 10 miscellaneous adults (line *j*). Based upon the statistics given in the second column of Table II.

The time ratios shown by the horizontal measurements and the dates expressed by the location of the vertical lines are as in Graph III. Note that by the end of the second year of adult life (line *i*) the body proportions are those of the average adult (line *j*).

entirely freeze over. Thus I have always found, even in midwinter, a little open space, sometimes only a few inches in width, and by breaking away the ice bordering the edges of this, have been able to reach the quiet pools under the ice, in which larvæ are found. Specimens have in this way been collected in January when the temperature was at 0° F. and had been at that point or lower for three or four days.

The larvæ frequent the quieter regions, always out of the current except when a temporary disturbance drives them into it. They seek not only quiet but very shallow water, where they lie motionless among the decaying leaves, which, owing to the wooded nature of their habitat, fill such pools. Such leaves also furnish quiet lurking places when they lie in a thick mass covering the surface of the more rapidly moving regions of the stream. Among the loose debris and sediment which these surroundings afford, the larvæ lie, their mottled brown color forming a perfect protective resemblance. The collector may thus at first stare several minutes into such a little pool before he recognizes in an apparent fragment of the midrib of a decaying leaf, the mid-dorsal ridge of a *Desmognathus* larva, and he may possibly realize the true nature of the object under observation only when upon disturbing the water, active swimming movements betray the living animal. On the other hand having once begun to recognize the larvæ one is frequently deceived into picking up some lifeless fragment which the larvæ so closely resemble. Several larvæ are frequently found in close proximity in the same little pool or mass of leaves, a fact which probably results from the abundance of the larvæ, but may in the early fall be due to the simultaneous arrival, in the same little pool, of several individuals of the same brood.

Frequently the larvæ, when concealing themselves among the leaves, lie half out of the water, the body immersed just sufficiently to bring the gills under the surface. This habit I have observed particularly in the case of individuals in captivity, and have thought that it might be associated with lack of sufficient oxygen. However the larvæ are certainly not adapted to the pressure conditions of deep water, and although they are quiet and apparently comfortable when in captivity in water a centimeter deep, if the depth of the water be gradually increased they show signs of uneasiness when it becomes about two centimeters, and by the time a depth of three or more centimeters is reached they make frantic efforts to reach the top of the water, where, however, they can sustain themselves only by active swimming or by resting upon some surface. In fact under these conditions they almost invariably swim to the edge of the aquarium, run rapidly up its side, and attempt to make their escape, while in shallow water they remain for days in an open dish and make no such attempt. This adjustment to shallow water is undoubtedly a means for insuring that they remain in regions where at the time of metamorphosis they can reach the necessary terrestrial environment; for the adults, being lungless, have no hydrostatic organs, neither have they any method of aquatic respiration other than the skin.

The larvæ react vigorously to attempts to capture them by any seizing act or any method which involves pressure against the opposite sides of the body, being evidently organized to escape from jaws and teeth. That they frequently barely succeed in making such escapes is evidenced by the large proportion of maimed individuals which one finds, tails and hind legs being the parts most frequently lost. The location of the lateral line organs or neuromasts upon opposite sides along the lateral surfaces of the body may have some significance in connection with this reaction. On the other hand, the larvæ do not appear to be especially sensitive to mere tactile stimuli even when these are applied to the regions where the neuromasts are most abundant, unless such stimuli are applied upon two opposing regions. After the application of such opposing stimuli, however, or the repeated application of single stimuli, the sensitiveness of the larva to touch seems to be for a time considerably augmented, and a stimulus applied to a single region will then give rise to vigorous efforts to escape.

On the other hand the larvæ appear to have no reflex mechanism for protection against forces which gently raise and lower the body, like the slight natural movements of quiet water. They may therefore be readily captured by lifting them with the hand, a watch glass, or any other concave object which may be passed gently under them and then quietly raised; the gradual flowing of the water from the surface upon which they are thus lifted produces also no disturbance so long as the larva remains moist. Larvæ may in this way be transferred from their natural habitat to the collecting jar, without a single movement on their part, while the slightest pinching or seizing force results almost invariably in such violent wriggling that the animal makes its escape.

The food of larvæ during the aquatic period consists mainly of little copepods and occasionaly very small aquatic insect larvæ. Mingled with the debris of such food in the stomach and intestine, there are usually found considerable quantities of sand and disintegrating vegetable matter, the ingestion of which is incident to the capture of the living food. The mouth opening does not extend very far back and it is equipped at each angle with a labial fold (*lf*, Fig. 17) so arranged that when the lower jaw is depressed the folds are stretched across the angles making the orifice almost circular in form and directed slightly downward. This is evidently an adaptation to the capture of such food forms as may be scooped up or sucked in from among the sediment at the bottom or upon the surfaces of decaying leaves.

The respiration during the larval period is accomplished by means of the three pairs of external gill bushes. Although these consist of relatively few filaments, they are held so widely outspread in the water as to render their position the most advantageous one possible to the performance of their function. When the larva is disturbed, however, as by opposing pressure applied to the lateral surfaces of the head, the gills are quickly drawn back and held closely appressed against the sides of the body, a protective reflex the efficiency of which is shown by the fact that one never finds larvæ with the gills injured.

With four gill slits upon each side, it would seem that the mechanism must be present for producing a flow of water through the mouth and pharynx and out over the gills, yet I have never been able to detect the slightest evidence of such a current, or of the rhythmic movements of the floor of the mouth and pharynx such as would be necessary to produce it. Occasionally there may be observed, however, a single, vigorous little movement of the gill bushes, which is undoubtedly for the purpose of assisting diffusion by hastening the change of the water in contact with the gills. This movement is usually performed once or twice when the larva comes to rest after vigorous swimming. The usual position of the larvæ when at rest, upon or among decaying leaves just below the surface of the water, gives natural access to the region of the water which is most completely aerated.

The gill filaments present a certain glistening white appearance which at times renders them the most conspicuous portions of the body. This appearance is similar to certain white spots which occur along the lateral surfaces of the body and occasionally in other regions, in both the larval and the adult stages, and have been described as "white pigment spots." There is considerable evidence, however, that in the gills, at least, the phenomenon is due to an accumulation of some gas, presumably carbon dioxide, in the tissues, but its exact nature and significance demand further investigation.

In the matter of internal structure as in size, the aquatic larva appears to be in a general static condition throughout the larger part of its larval life. Late in the spring, however, shortly before the metamorphosis, a series of changes becomes inaugurated, involving especially the integument and the organs derived from it, as well as certain skeletal parts, and leading rapidly to the conditions which characterize the adult. Such changes are to be considered as belonging particularly to the period of metamorphosis, and will be described as such.

Until such distinctive metamorphic changes appear, the skin of the larva remains in practically the condition attained at the end of the terrestrial period. The two layers of cells of the epidermis remain fairly well defined. The cuticular border of the outer layer becomes thicker and more compact in appearance, and the cap of intracellular pigment between this border and the nucleus becomes denser. In the deeper layer, the cells of which rest directly upon the dense corium, the distended vacuolated Leydig cells are in most regions so abundant that each is usually separated from the neighboring Leydig cells by but a single circle of cells of the general epidermal type (Plate IV., 25). Here and there among the cells of the deeper layer are cells which are considerably elongated and somewhat piriform and which extend out between the cells of the outer layer almost to the external surface. These are the cells which in the German literature are designated as the "Schaltzelle" (Plate IV., 23, *intr*). As the larval period advances there is a gradual increase in the thickness of the epidermis due to the increasing size of the cells of the deeper layer, especially the Leydig cells, which gradually become more distended, while their nuclei become notice-

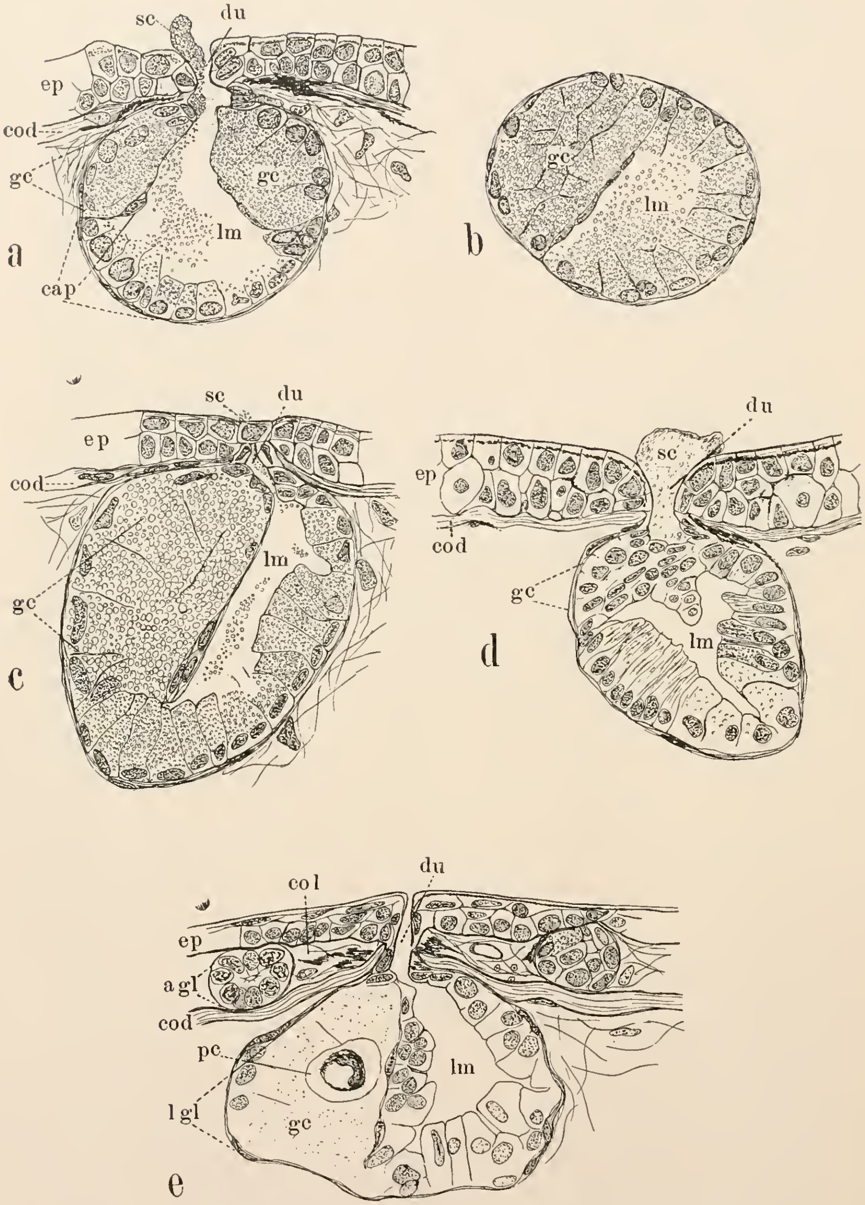

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FIG. 15. Sections through larval acinous glands; (*a*) vertical section, *i. e.*, at right angles to the external surface, through the middle of a gland of the suprabranchial group of a recently hatched terrestrial larva; (*b*) cross section, *i. e.*, parallel with the external surface, through the middle of one of the mid-dorsal

ably smaller. The nuclei of the cells of both layers appear in many cases irregular in form and often show sharp constrictions which suggest amitotic division, a suspicion further confirmed by the increasing number of multinucleate cells which, in spite of the absence of mitotic phenomena, appear in the outer layer of the epidermis, particularly in the latter part of the larval period. As the Leydig cells become larger with the continuance of larval life, some of the surrounding cells of the deeper layer become crowded together into a columnar form, while others appear to be pushed out of line, some lying in the deeper region of the epidermis and others becoming crowded into the angles between the bases of the cells of the outer layer (Plate V., 29). Thus the number of cells which reach from the deeper layer into the outer layer becomes increased. These, however, seem not to actually belong to the external layer since, even if they reach the surface, they apparently do not acquire a cuticular border. These slight changes in the epidermis are accompanied by a gradual increase in the thickness of the dense corium, from which, moreover, connective tissue cells gradually invade the epidermis. These together with leukocytes and branches of nerve fibers may thus be seen among the cells of the deeper layer, and not infrequently these invaders, as well as the pigment cells, actually break through the thin membrane of the Leydig cells, and lie within the large vacuolated spaces which these afford.

The acinous glands (Fig. 15), which have already developed and begun to function at the time of hatching, remain in a fully

group of glands of a terrestrial larva; (*c*) vertical section through one of the glands of the supra-branchial group of an aquatic larva in late spring, hence near the end of the larval period; (*d*) vertical section through one of the supra-branchial group of an aquatic larva in February, showing an almost completely discharged condition induced by prolonged mechanical stimulation immediately before the specimen was killed; (*e*) vertical section through one of the supra-branchial group of an individual immediately after metamorphosis, showing the gland in an empty condition and undergoing atrophy, while in the loose corium nearby are seen two of the newly developed acinous glands of the adult period of life. Note the position of the larval gland below the level of the dense corium, while the adult glands are in their permanent location external to this layer.

Agl, adult gland; *cap*, capsule of the gland; *cod*, dense corium; *col*, loose corium; *du*, duct of the gland; *ep*, epidermis; *gc*, giant cells of the gland; *lm*, lumen of the gland; *pc*, pigment cell which has invaded the gland; *sc*, secretion discharged from the gland. Drawn with Abbé camera. × 225.

active condition throughout larval life. These glands, arranged as above described (cf. p. 291 Vol. XXIV. *Biol. Bull.*), lie quite below the dense corium, embedded in the subcutaneous connective tissue, and communicating with the external surface by means of a narrow duct. Although of the type of amphibian gland variously designated as "granular" or "poison," they do not in detail correspond in all respects to the descriptions of such glands in other amphibians. They vary greatly in size, the largest when in a distended condition having a diameter eight times the thickness of the skin, while the smallest when distended have a diameter only twice the thickness of the skin. They are spherical or subspherical in external form; the larger ones, especially those of the suprabranchial group, are not of the simple acinous type which this external form suggests, however, since internally the lumen is partially subdivided by invaginations of the single layer of gland cells, as well as by the occurrence here and there of groups of these cells which are much taller than the rest and thus encroach upon the lumen. The connective tissue sheath (*cap*) of the gland follows closely the invaginations. The glands of all sizes are particularly characterized by the development of certain cells into the "giant" type (*gc*) through an enormous accumulation of granules of the same sort apparently as those which fill the other cells of the gland. These giant cells are differentiated very early in the development of the glands, even while mitosis is still in progress and before the lumen of the gland has appeared; in all of the glands they comprise at least one group of some eight or ten cells which lie near the orifice of the gland upon one side and become almost completely surrounded and cut off from the rest of the gland by the ingrowth of the connective tissue sheath (Fig. 15, *b*). Thus placed they form a mass nearly equal in volume to half of the whole gland, so that a section through the middle of the gland parallel with the external surface of the skin often shows the remainder of the gland wrapped in a crescentic form about this mass of giant cells. Giant cells occur also in other parts of the larger glands, either as isolated cells or in small groups of two or three. The granules filling all of the gland cells are similar, except that those in the giant cells become much larger. The ordinary cells, however, appear to constantly discharge their contents into the lumen of the gland, where they form into a fluid which may often be seen in the sections in the process of being discharged through the gland duct. The discharge of the granular contents of the gland cells seems to involve a rupture of the cell membrane. The giant cells, on the other hand, seldom appear to be discharging their granules even when the remainder of the gland shows great activity, although the membranes of a group of such cells not infrequently appear to be ruptured, and a large accumulation of granules to be ready to be discharged. When, however, a larva is killed after having been subjected to much mechanical stimulation in the nature of poking and seizing with the forceps, not only are the ordinary gland cells found empty or nearly so, but the giant cells also are empty and the mass of cells, separated from the rest of the gland by its sheath, is collapsed (Fig. 15, *d*). It thus seems that these giant cells are in the nature of a reserve supply, ready for instant response in an extreme emergency when the constant secretive action of the ordinary gland cells is inadequate. The only other possible explanation is that the secretions of the ordinary and the giant cells are different in function, a supposition which may upon further investigation prove to be correct, although the appearance of the secretions within the cells gives no indication of such a difference.

The condition of the nuclei of the gland cells in the fully discharged condition does not in the least indicate that the cells are doomed to degeneration; the nuclei are large and round and appear fully vigorous, much like the nuclei of the epidermal cells. Neither does it appear that there is any provision for the replacement of glands during the larval life, either in the form of single replacement cells or the anlagen of new glands; and, as all the glands, both large and small, are equally mature and active and exist in practically the same numbers throughout the whole larval life, there is no chance for the supposition that the small ones grow into the large ones. We are forced, then, to the belief that the larva hatches fully equipped with a set of glands of the granular type that are to perform their function without renewal throughout the many months of larval life.

That this function is important is very evident, though its exact nature is a problem. The usual explanation of the function of the granular type of gland in the Amphibia is that its secretion is of a poisonous nature and protects the animal from capture by other forms which might use it as food. The full discharge of the contents of the glands by the *Desmognathus* larva under prolonged mechanical stimulation seems to corroborate this explanation, as does also the special supply of these glands in the region of those extremely important organs, the gills. On the other hand, the enormous development which this type of gland reaches in forms like *Bufo* and *Plethodon*, which have become very terrestrial, seems to indicate a correlation, at least, with some condition incident to terrestrial life, a conclusion further substantiated by the fact that the metamorphosis to the adult state in *Desmognathus* involves the development of a great multitude of glands of the granular type distributed over the entire surface of the body, in place of the few isolated groups of such glands with which the larva is supplied. Further, the fact that so closely related a form as *Spelerpes bilineatus* does not possess such glands while in the larval state leads one to ask what difference in environment or habits of these two species could serve to explain so decided a structural difference. We at once remember that the most essential difference lies in the fact that *Desmognathus fusca* is terrestrial in its egg laying habits while *Spelerpes bilineatus* is aquatic, and that consequently there is no terrestrial larval period in the latter species. Moreover, *Spelerpes* larvæ live in deeper pools than do the *Desmognathus* larvæ, and so far as I have observed, do not have the habit of lying upon leaves at the very surface of the water, which is so characteristic a position of the *Desmognathus* larvæ, and one which brings out of the water exactly those regions of the body which are supplied with the acinous glands, the mid-dorsal region of the trunk and the slightly elevated latero-dorsal surfaces of the head. Here again, then, there seems to be a decided connection between exposure to air and the function of the granular acinous glands. The solution of the problem is one which can be made only after extended experimental comparative study of different amphibians and must therefore be postponed until such study is completed.

The other set of specialized integumental organs, the neuromasts, are also prominent features of the skin during the entire larval life. They remain in practically the same condition except that as the skin becomes thicker and more compact, the accessory cells of the neuromasts become more numerous and more closely crowded together. Organs of this type are said to be associated with aquatic life and to be adapted to the reception of pressure stimuli. In view of this, it is interesting to note that the neuromasts are probably connected with the ready response of the larva both to a slight increase in the depth of the water above it, and to mechanical pressure applied to opposite sides of the body.

METAMORPHOSIS.

Metamorphosis occurs, as above stated, in late spring. The earliest date at which I have collected larvæ showing signs of the approaching change is May 11, the latest is June 17. There is strong evidence, however, in the histological conditions and body proportions of the small adults of from 33 mm. to 35 mm. in length which I have collected early in June, that they have only recently undergone metamorphosis, and adult specimens of similar size and proportions may be collected in early fall also (Table II. and Graphs III. and IV.). It thus seems probable that the period of metamorphosis may begin as early as May and may extend well into the summer, a range of time comparable to that known to exist for both the egg-laying and the hatching periods.

Larvæ which are undergoing metamorphosis are usually not found in the water but among the dead leaves and loose debris along the edge, often headed away from the water as if they were in the act of crawling out of it.

The noticeable changes in external appearance which take place during the process are the following:

1. The body proportions change, the head becoming relatively shorter and its posterior region narrower and the tail longer.

2. The median fin fold characteristic of the aquatic larval period undergoes atrophy, the cross section of the tail thus acquiring a more rounded contour.

3. The gill bushes gradually diminish in length, each filament becoming blunt at its extremity instead of slender and pointed,

and finally the gills entirely disappear, a process involving the final closure of the gill slits.

4. The whole gill region becomes shorter and narrower, the latter change being due in part to the flattening down of the prominent bulging supra-branchial regions so that this portion of the head is no longer the widest one.

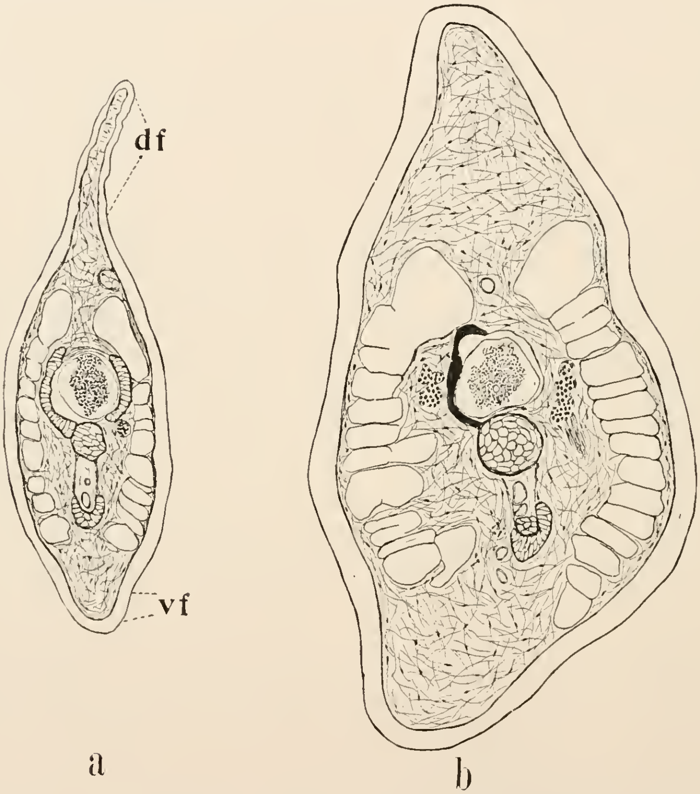

Fig. 16. Cross sections at the level of the 10th vertebra posterior to the cloaca showing (*a*) the typical aquatic larval form of tail with the dorsal (*df*), and the beginning of the ventral (*vf*) fin fold; (*b*) the change of form at the approach of metamorphosis. Note that (*b*) is cut slightly obliquely. Drawn with Abbé camera. × 60.

5. Further striking changes in the appearance of the head are due to the development of eyelids (*eyl*, Fig. 17) and of glandular masses in the orbit ventral to the eye, so that the eye and its surroundings come to possess the bulging appearance which is so characteristic an adult feature.

6. The naso-labial groove (*gr*, Fig. 17) makes its appearance, extending from the latero-ventral border of each external naris until finally, at the completion of metamorphosis, it has reached the border of the upper lip.

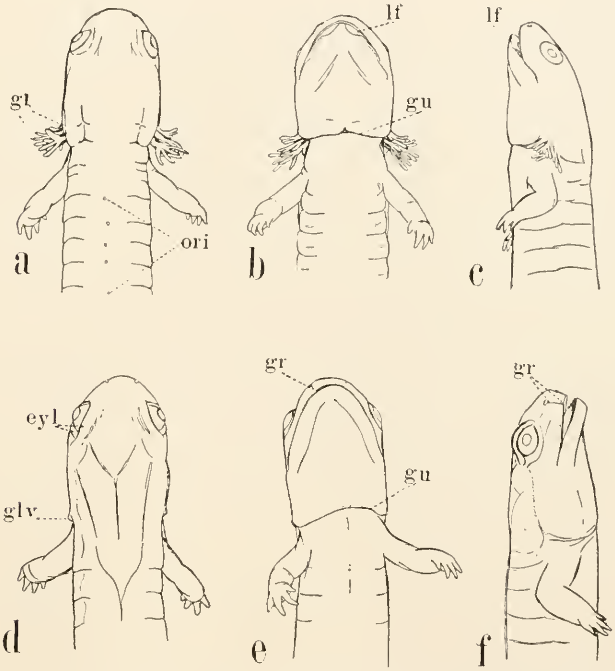

FIG. 17. (*a*), (*b*), and (*c*), dorsal, ventral, and lateral views respectively of the anterior region of the body of a *Desmognathus fusca* larva in which metamorphic changes have begun to appear; (*d*), (*e*), and (*f*), dorsal, ventral, and lateral views respectively of the anterior region of the body of a *Desmognathus fusca* in which the process of metamorphosis is just completed; *eyl*, eyelids, present only after metamorphosis; *gl*, gills which have become slightly blunted and shortened in (*a*), (*b*), and (*c*), and have disappeared altogether except for a slight vestige, *glv*, at the time of metamorphosis; *gr*, nasolabial groove, present only after metamorphosis; *gu*, gular fold; *lf*, labial fold which disappears at metamorphosis; *ori*, orifices of some of the larval acinous glands. Note that with metamorphosis the posterior region of the head becomes relatively narrower. Drawn with Abbé camera. × 4.25.

7. The labial folds (*lf*, Fig. 17) disappear, and the extent of the mouth opening increases, the lateral angles coming to lie as far back as the posterior angle of the orbit; thus a form of mouth opening adapted to the capture of larger prey, from a terrestrial as well as an aquatic medium, supplants the scooping or sucking apparatus of the aquatic larva.

8. The whole external surface gives evidence of the formation of a thin moult layer of epidermis, which here and there becomes separated from the deeper layers and thus presents a loose appearance.

In individuals in captivity these changes in external appearance have been observed to occur in the course of a few days during which time the individual crawls persistently out of the water. The metamorphosis may be considered quite complete when, in addition to the above enumerated changes in external appearance, the distinctive physiological metamorphic phenomenon occurs, and the animal, with mouth tightly closed, lowers the floor of the mouth and pharynx, and, drawing in air through the short nasal passages, fills its bucco-pharyngeal cavity and thus establishes aerial respiration.

Although the final steps in metamorphosis take place with great rapidity, the histological changes preliminary to the process begin considerably earlier. Thus specimens of larvæ collected in May show decided indications of the approaching change. The most striking of these indications is the large amount of mitosis which is in progress especially in the deeper layer of the epidermis (Plate V., 26, 27 and 29), resulting in so rapid a multiplication of the epidermal cells that the earlier arrangement in two fairly definite layers gives place to a considerable irregularity of arrangement of the cells of the deeper layer, the epidermis thus coming practically to consist of three layers. The arrangement of the cells of the outer layer remains unaltered, however, their cuticular borders forming, as before, a continuous external covering. The cells of the outer layer become, however, more and more flattened and are eventually to be cast off as the first moult layer, not, however, until after metamorphosis.

This flattening of the external layer of cells in part accounts for the actual decrease in thickness of the epidermis which becomes evident as the time of metamorphosis approaches; by far the greater cause of this, however, lies in the gradual disappearance of the Leydig cells, which by their turgor so greatly increase the thickness of the epidermis during early larval life. The invasion of these cells by pigment cells, connective tissue cells and leukocytes during the larval period has already been noted. As metamorphosis approaches (Plate V., 28, 29, and 30), the nuclei of the Leydig cells (*lc*) become shrunken and the cell space is finally entirely given over to the intruding elements (*pgc(inv)* and *leu*) from the deeper region. The surrounding cells of the deeper epidermal layer, although increased in number by mitosis so as to form practically two layers instead of one, are correspondingly decreased in size and thus do not together equal in thickness the former diameter of the Leydig cells.

The thickness of the whole skin, *i. e.*, epidermis plus corium, does not become less; for coincident with the decrease in thickness of the epidermis there is a formation of loose vascular corium (*cor.l*) external to the dense corium and separating from it the deeper layer of epidermal cells which are in contact with it during the larval period. The formation of this loose corium is indeed inaugurated in the migration of leukocytes and connective tissue elements into the vacuoles of the Leydig cells and between the cells of the epidermis. But it is only with the breaking up of these Leydig cells in preparation for metamorphosis that these elements and other similar ones come to form a definite layer, and even then many of the connective tissue elements intrude themselves between the epidermal cells.

The development of integumental glands is particularly characteristic of the preparation for metamorphosis. These glands may be enumerated as follows: (1) General acinous glands; (2) naso-labial glands; (3) orbital glands.

The acinous gland anlagen appear during the general premetamorphic mitotic period of the epidermis (Plate V., 29 and 30). They arise from the deeper layer of the epidermis and may first be distinguished as little groups of from four to eight cells with large nuclei, some of which are usually in the process of mitosis (*ac*, Plate V., 29). They are abundantly distributed over practically all regions of the body, although somewhat less numerous in the skin covering the appendages. They rapidly increase in size and assume the form of hollow spherical acini, each with a definite lumen which opens to the exterior by a narrow duct (Plate V., 30). The bodies of the glands intrude into the loose corium and thus lie below the level of the epidermis, which is, however, thin in the region immediately surrounding the gland. Embedded thus in the loose vascular corium, each gland comes to be surrounded by a capillary ring (*cap*), which, joining similar rings about the neighboring glands, forms a part of the rich capillary network of the skin. These glands and their ducts, the latter slender and lined with thin cells which are continuous with the cells of the external layer, are fully formed at the time of metamorphosis and before the first moult (*ml*, Plate VI., 31) occurs. The nature and function of their secretion and the methods by which they are replaced must be left for further and more extended investigation. Suffice it to say that many, if not all of them, show from the first a striking similarity in structure to the relatively larger and less numerous acinous larval glands above described. One anatomical distinction between the larval and adult glands must, however, be clearly understood, namely, that the larval glands lie wholly *below* the level of the dense corium, the ducts alone passing through this to reach the external surface. The larval glands remain functional up to the time of metamorphosis, and thus in the metamorphosing larva both the larval and the adult sets of glands are present, the former beneath the dense corium, the latter external to it within the loose corium (cf. Fig. 15, *e*). After metamorphosis, however, the larval glands collapse and lose their connection with the external surface, their cells undergo atrophy, and the glands rapidly disappear. It is this atrophy of the supra-branchial group of glands which results in the marked narrowing of this region of the head at metamorphosis (cf. Fig. 17).

The naso-labial glands consist of a series of tubular glands opening in close proximity to the external nasal orifices and along the border of the naso-labial groove (Whipple, '06). Of these glands (many of which become very extensive in the fully developed adults) the two largest (nl_1 and nl_2, Figs. 18, 19, and 20) begin their development before metamorphosis, and at the time of metamorphosis have attained a size and condition indicative of their functional importance in adult life. The one of these which opens at the dorsal angle of the nasal orifice extends, at this time, beneath the skin as far posteriorly as the anterior angle of the orbit, is somewhat convoluted, and branches near its distal (posterior) end; the other gland, which opens near the ventral angle of the naris, is also well developed, extends nearly as far posteriorly as the first but in a more ventral location, and as yet shows no branching. Although other glands of the group are slightly developed, none of the tubules have as yet become so extensive as to enter either the premaxillary foramina or the grooves of the maxillary bones as they are to do later in the fully developed adult. Simultaneously with the development of these naso-labial glands the muscular apparatus for opening

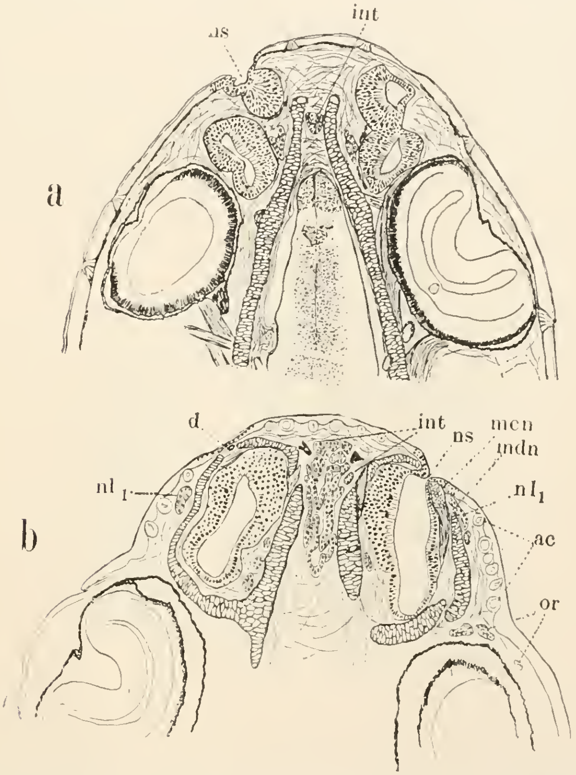

FIG. 18. Horizontal sections through the nasal region of (*a*) *Desmognathus fusca* larva collected in February, and (*b*) an individual immediately after the completion of its metamorphosis, for comparison of the glandular development; *ac*, acinous glands of adult stage; *d*, duct of the most dorsal of the naso-labial glands (nl_1); *int*, intermaxillary glands; *mcn*, and *mdn*, musculus constrictor naris, and musculus dilatator naris respectively; nl_1, the first nasolabial gland; *ns*, external naris; *or*, orbital glands. Drawn with Abbé camera. × 32.5.

and closing the naris develops. This consists of a constrictor and a dilatator muscle (*mcn* and *mdn*) and forms an effective apparatus very characteristic of adult amphibians (Bruner, '96 and '01, I. W. Wilder, '09).

The naso-labial glands have to do with the terrestrial life into which the process of metamorphosis introduces the animal, in that they not only keep pliable the thin crescentic fold which by means of the contraction of the constrictor muscle closes the external naris against the entrance of foreign bodies, such as dirt when the animal is burrowing, but they also appear to have the peculiar function of so repelling water from the surface of the skin immediately surrounding the naris and bordering the naso-labial groove that the latter may, by capillary action, almost instantaneously drain off the tiny drop which fills the nasal depression after a temporary immersion in water. Thus this water is prevented from entering the nasal passage when the animal, upon emerging from the water, reopens the nares by withdrawal of the crescentic fold. The nasal passages are by this means kept dry and fully

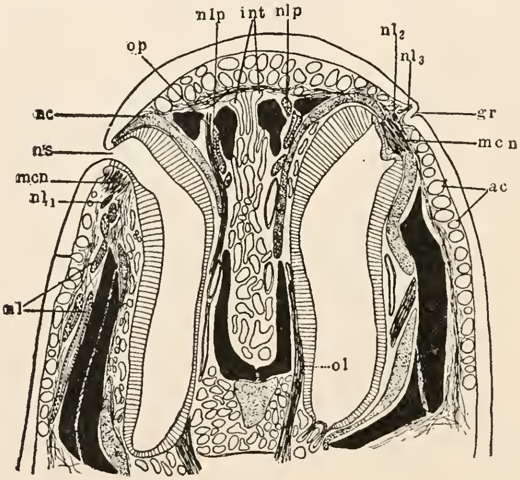

FIG. 19. Horizontal section through head of adult *Desmognathus fusca*, × 20. *Ac*, acinous glands of skin; *gr*, cross-section of naso-labial groove; *int*, intermaxillary glands; *mcn*, M. constrictor naris; *nc*, nasal capsule; *nl*, naso-labial glands; *nl*$_1$, *nl*$_2$, *nl*$_3$, the first, second and third naso-labial glands respectively; *nlp*, tubules of naso-labial glands within the premaxillary foramina; *ns*, external naris; *ol*, olfactory nerve; *op*, internal nasal branch of ophthalmic nerve.

ready for the immediate resumption of aerial respiration (Whipple, '06). It is a significant fact, therefore, that at the completion of metamorphosis this naso-labial apparatus is in working order.

The orbital glands lie ventral to the eyeball (*or*, Fig. 21, *b* and *d*)and open along the depression between the inner surface of the lower eyelid and the eyeball itself. They are tubular glands and make their appearance simultaneously with the formation of the fold which gives rise to the lower eyelid itself, shortly before metamorphosis.

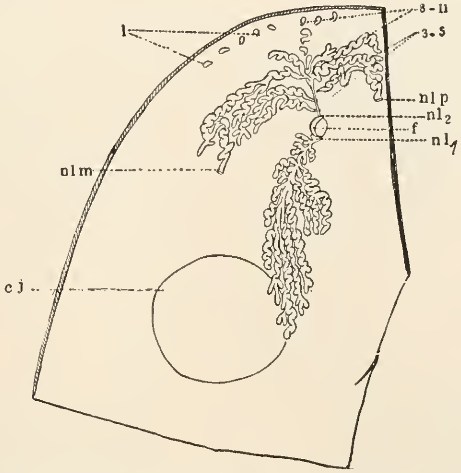

FIG. 20. Dissection of adult *Desmognathus fusca* in which the skin is removed from the right side of the head, the naso-labial glands being removed with it. Drawing shows under surface of skin with glands in place. *Cj*, conjunctiva; *l*, labial glands; 3–5 and 8–11 indicate the enumeration of the naso-labial glands; *nlm*, cut end of tubule which lies in the groove of the maxillary bone. Other designations as in Fig. 19.

In connection with the development of the orbital glands it should here be noted that unlike all other Urodeles (so far as the facts have been reported), *Desmognathus* has no naso-lacrimal ducts. As these are present in so closely related and associated forms as *Spelerpes* and *Plethodon*, as well as in the less closely related lunged forms, their absence here has some significance which demands further study of the comparative morphology and the habits of this species.

In addition to the integumental glands, the development of two other sets of glands must be noted among the premetamorphic changes, the intermaxillary and the lingual glands. The former is an unpaired gland and is present indeed throughout larval life (*int*, Fig. 18) as a single, slightly convoluted tube

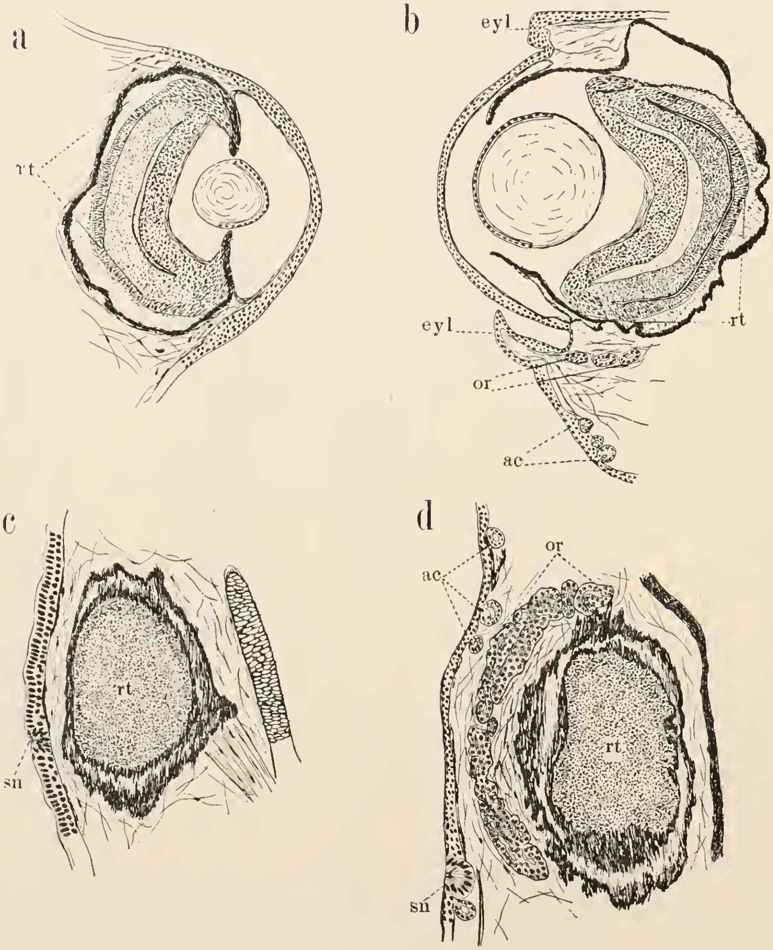

FIG. 21. Sections for comparison of the eye and its surroundings in the larval and adult stages of *Desmognathus fusca*; (*a*) transverse section through the left eye of a larva; (*b*) transverse section through the right eye of a recently metamorphosed adult; (*c*) horizontal section through the ventral region of the eye of the larva; (*d*) horizontal section through the ventral region of the eye of a recently metamorphosed adult. *Ac*, acinous glands; *eyl*, eyelids; *or*, orbital glands; *rt*, retina; *sn*, sense organs of the integument. Drawn with Abbé camera. × 60.

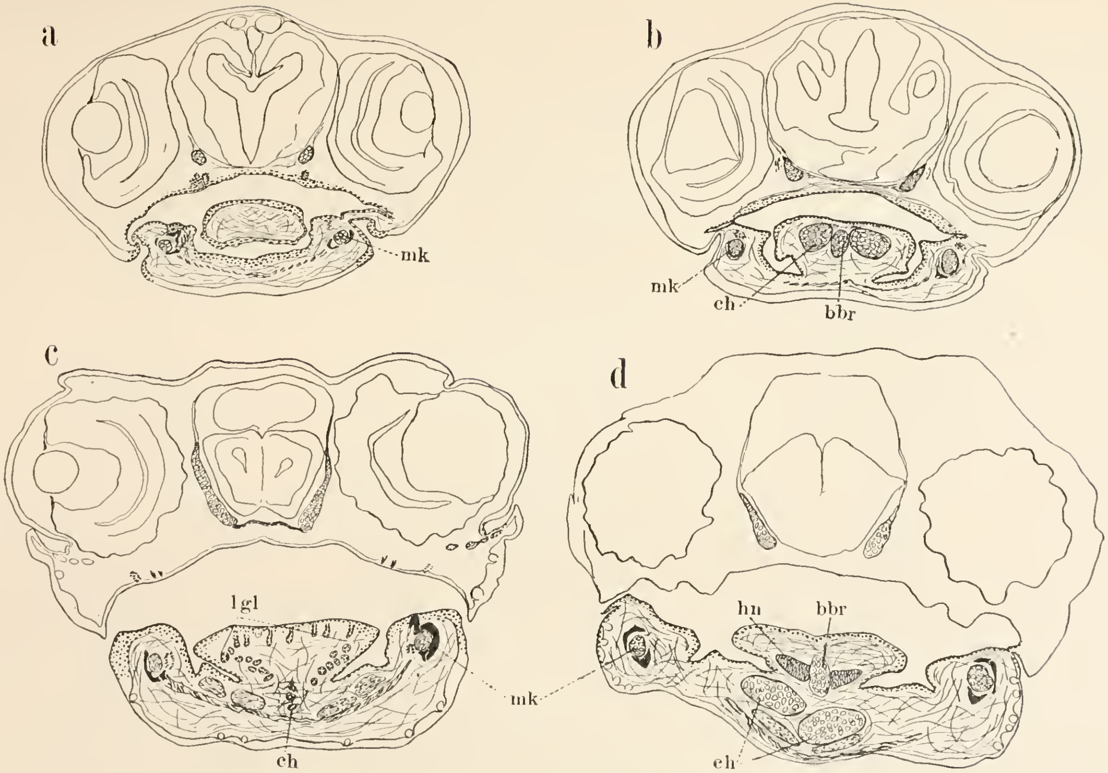

FIG. 22. Cross sections through the heads of (*a*) *Desmognathus fusca* larva at the level of the free anterior region of the tongue, (*b*) the same larva at the level of the articulation of the ceratohyals and basibranchial (cf. Fig. 25, *a*), (*c*) recently metamorphosed adult at a level corresponding to (*a*), and (*d*) the same adult at the level corresponding to (*b*) (cf. Fig. 25, *b*); *bbr*, basibranchial; *ch*, ceratohyal; *hn*, accessory horns; *lgl*, lingual glands; *mk*, Meckel's cartilage. Drawn with Abbé camera. × 32.5.

leading dorsally from the roof of the mouth, from a point a little anterior to the posterior nares. During the premetamorphic period of mitosis and glandular development, the intermaxillary gland becomes enormously enlarged and complicated and fills the space between the two maxillary bones with its mass of convoluted tubules.

The tongue possesses no multicellular glands until, with the approach of metamorphosis, large numbers of tubular glands (*lgl*, Fig. 22, *c*) appear opening in the more distal region of the tongue upon its dorsal surface, and transforming the tongue into a glandular mass. The development of both intermaxillary and lingual glands at this time is, of course, suggestive of a preparation for the terrestrial life of the adult. By means of their secretions the mouth is kept moist both for its respiratory function and to render more sure the capture and retention of terrestrial prey.

Among the most significant of the structural changes involved in metamorphosis are those concerned in the atrophy of the gills and the modifications of their associated structure, the visceral skeleton. Externally changes do not occur in the gill bushes themselves until within a few days of the completion of metamorphosis. Then the filaments become shorter and more rounded, and the whole structure undergoes rapid shrinkage and atrophy. As the gills are merely blood vessels covered with a thin layer of epidermic cells, the withdrawal of the blood from them so reduces them in size that the process of atrophy can take place very quickly.

The metamorphic changes in the visceral skeleton consist mainly in the atrophy of the distal end of the first epibranchial and the atrophy of the whole of the second, third, and fourth epibranchials (Figs. 23, 24, and 25). The process begins at the time of the premetamorphic mitotic period, and appears first in the distal ends of the epibranchial cartilages where the hyaline matrix becomes dissolved around the groups of cartilage cells (Fig. 23, *b*). With the atrophy of the free ends of the epibranchials, there occurs that shortening of the gill region which is mainly responsible for the decrease in the proportionate length of the head as the animal passes from larval to adult life. Simul-

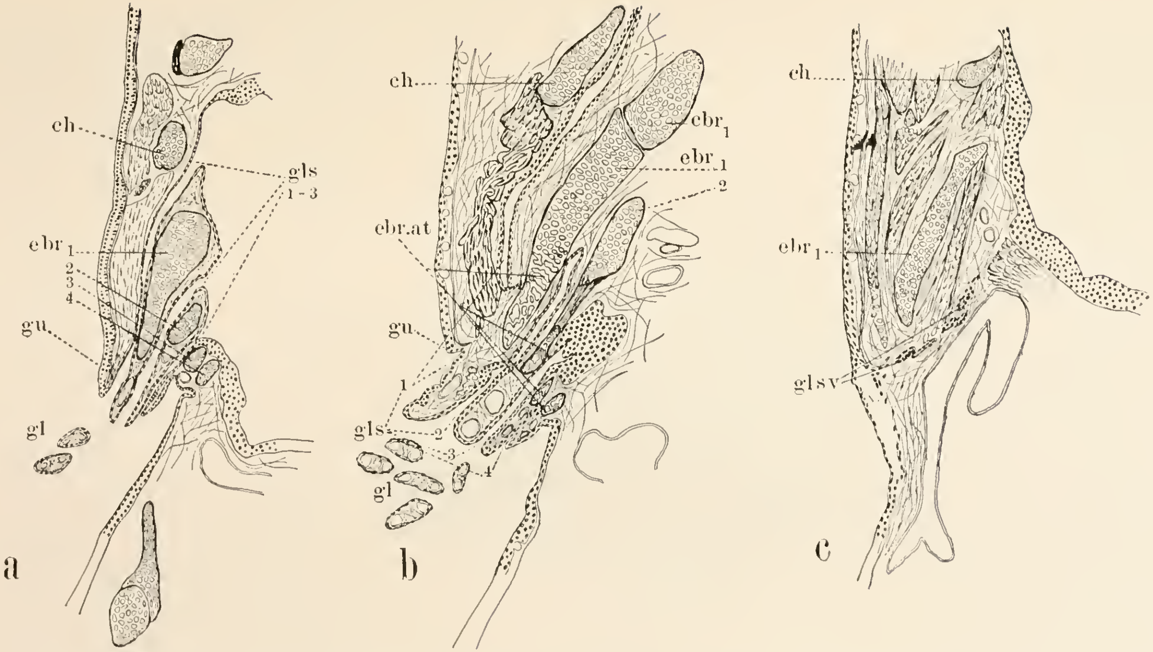

FIG. 23. Horizontal sections through the gill region of *Desmognathus fusca* illustrating the changes which take place at metamorphosis; (*a*) aquatic larva collected in February, showing the typical larval condition of the region; (*b*) larva in which metamorphic changes have begun (cf. Fig. 17, *a*, *b*, and *c*); (*c*) an individual which has just completed its metamorphosis (cf. Fig. 17, *d*, *e*, and *f*); *ch*, ceratohyal cartilage; *cbr*1, first ceratobranchial cartilage; *ebr 1*, *2*, *3*, and *4*, epibranchial cartilages; *ebr.at*, regions of the epibranchials which are undergoing atrophy; *gl*, gills; *gls*, gill slits; *glsv*, vestiges of the gill slits; *gu*, gular fold. Drawn with Abbé camera. × 32.5.

taneously with the atrophy of the epibranchials there occurs an atrophy of the whole of the second basibranchial except its extreme posterior end, which persists and later ossifies to become a little bifurcated structure designated by Wiedersheim ('77) as the thyreoid bone (*bbr 2*, Fig. 25, *b*). With the atrophy of the second basibranchial cartilage, which during larval life forms one continuous chondrification with the first pair of ceratobranchials, the latter become separated from each other and each articulated with the first basibranchial. In the two or three final days before metamorphosis the medial ends of the ceratohyals become detached from the anterior ends of the first basibranchial (*ch*, Fig. 22, *b* and *d*), while posterior to their former point of attachment, a pair of cartilaginous processes grow out from the basibranchial to serve apparently as an additional support for the tongue (*hn*, Figs. 22, *d*, 24, and 25).

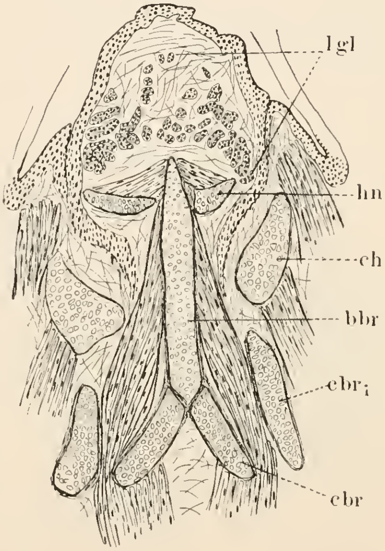

FIG. 24. Horizontal section through the tongue and hyo-branchial apparatus of a recently metamorphosed adult *Desmognathus fusca* (cf. with the drawing of the whole apparatus shown in Fig. 25, *b*); *bbr*, basibranchial; *cbr 1* and *2*, first and second ceratobranchials; *ch*, ceratohyal; *hn*, accessory horns for the support of the adult tongue; *lgl*, lingual glands. Drawn with Abbé camera. × 32.5.

The final change which completes the atrophy of the gill region is the closure of the gill slits of which there are four upon each side, the most anterior being the extensive one anterior to the first gill arch and beneath the gular fold. Previous to metamorphosis the gular fold becomes noticeably shortened, keeping pace with the shortening of the cartilaginous arches.

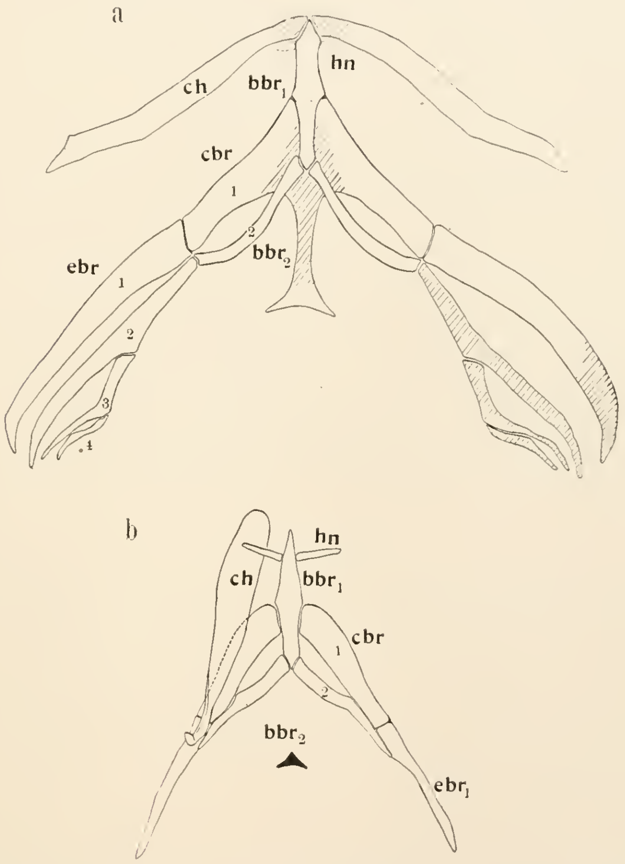

FIG. 25. (*a*) A dorsal view of the hyo-branchial apparatus of an aquatic larva collected in mid-winter, showing the typical larval structure. The shading indicates the regions which will atrophy at metamorphosis. The dotted lines indicate the regions where the accessory horns (*hn*) will develop at the time of metamorphosis. *Bbr*$_1$ and *bbr*$_2$, first and second basibranchials; *cbr*$_1$ and *cbr*$_2$, the first and second ceratobranchials; *ch*, ceratohyal; *ebr 1*, *2*, *3*, and *4*, the four epibranchials. Drawn with Abbé camera from a preparation stained *in situ* with methylene blue, and cleared with clove oil. × 40.

(*b*) A dorsal view of the hyo-branchial apparatus of an adult *Desmognathus fusca*. Designations as in (*a*). Note the persistence and ossification of the posterior end of the second basibranchial, forming the *os thereoideum* of Wiedersheim ('77). Drawn with Abbé camera from a dissection. × 6.

With the final withdrawal of the blood from the gill bushes, the latter lie against the lateral wall of the pharyngeal region like a temporary operculum, and rapidly fuse with the epidermis covering the surface over which they lie, thus effecting the external closure of the gill slits (Fig. 23, *b* and *c*).

For some time after metamorphosis the whole region is in a disorganized condition histologically, and sections show beneath an external covering of the thinnest of epidermic layers, a great meshwork of distended blood vessels, together with the remnants of epithelial lining of gill slits, which through the agency of the ever active leukocytes is rapidly disintegrating.

The transition from larval to adult life involves in *Desmognathus* no fundamental change in the nature of the food, since throughout the whole life of the species the food consists mainly of living animal forms. As would be expected, therefore, metamorphosis necessitates no noticeable changes in the digestive apparatus, as it does in the case of those amphibians in which the nature of the food changes at metamorphosis from vegetable to animal.

Adult Stage.

With regard to the growth and development of *Desmognathus* subsequent to metamorphosis I can give no very exact data. The many adults which I have measured range from 29 mm. to 104 mm. in length, the former showing, of course, unmistakable evidences of recent metamorphosis. Specimens collected at any one time do not show sufficient evidence of falling into distinct groups as to size to suggest definite yearly amounts of growth, with the possible exception of the smaller sizes which, in the case of those collected in the late spring, for example, fall into groups averaging 33 mm., 43 mm., and 56 mm. in length, the former recently metamorphosed, and the others presumably adults of one and two years' growth, respectively. Since the proportionate lengths of body regions of the smallest adults show a much wider range of variation as well as a closer similarity to the proportions of the larvæ than to those of adults of 55 mm. and over, it seems evident that not only is the growth during the first year or two more rapid but that the change of length proportions from those at metamorphosis to those of the final adult life, a change which involves a relatively more rapid growth of the caudal than of the other regions, also takes place more rapidly during the first two years (cf. Table II., Graphs III. and IV.). All of the adults, however, show a slight range of variation in the proportions of length of body regions which is in general correlated with the size of the specimens, as if the caudal region continued always to grow slightly more rapidly than the rest of the body. Here, however, the actual facts are sometimes masked by the frequency of regeneration of the tail, since a large percentage of adults show the tail to be in some stage of regeneration. I have seen no evidence that this loss of tail is due to self-mutilation as in the case of *Plethodon*, although the natural supposition would be that such is the case. Such regenerating specimens have, when detected, been excluded from my statistics, but when the process of tail regeneration is nearly complete, it is not always possible to decide whether the specimen presents a case of regeneration or one at the lower limit of normal variation. The question of the time of sexual maturity may also throw some light on the duration of the period of the more rapid growth of adults. At the time of metamorphosis the two sexes may be distinguished by the histological condition of the germ cells, some of the ova having already entered upon the growth period. Small adults, up to the 43 mm. stage, *i. e.*, presumably of a year's growth as adults, give no indication of sexual maturity. I have had too little opportunity to examine in this particular, specimens of the next larger size, presumably of two years' growth, to be able to state definitely that they may not be sexually mature at this time, but the few that I have examined were not so. On the other hand, specimens of 68 mm. and over of both sexes are sexually mature, apparently after three years of adult life. In the mating season the lips of the cloaca are often somewhat everted, and the two sexes may be distinguished by the fact that the female cloacal lips display numerous folds which converge anteriorly, with an anteriorly projecting papilla in the mid-line at the posterior angle of the two lips. In the end of this papilla, which lies within the cloacal cavity, is the orifice of the spermatheca. The lips of the male cloaca are thickly covered with villi-like processes.

The changes in structure during adult life are otherwise those of the natural growth of the various organs. Thus, although the epidermis retains the condition reached soon after metamorphosis of some two or three layers of cells beneath the moult layer, the acinous glands increase constantly in size and number, and the loose corium in which they are embedded, as well as the dense corium beneath, becomes correspondingly thicker and the whole skin more deeply pigmented and more vascular. So also the other integumental glands, such as the naso-labial and the orbital, become more numerous and their tubules more convoluted and longer, while the naso-labial groove itself grows deeper and more clearly marked.

The muscular development keeps pace with the general increase in size. Thus the power and rapidity of motion which may be observed in all stages of this species becomes very pronounced in the large adults. The long muscular tail plays an important rôle in locomotion, acting as a strong propelling organ as it strikes against the ground first upon one side, then upon the other, thus sending the body forward. This method of locomotion is of course a part of the adaptation to a burrowing habit.

The following list of the contents of the stomachs of 18 specimens of adult *Desmognathus* of different sizes and collected at different times and from different localities will serve to illustrate the variety of food materials which are made use of. It will be noted that the food is wholly animal and that the list includes aquatic as well as terrestrial forms.

Specimen No. 1. Moulted skin of *Desmognathus* (presumably his own).
No. 2. A caddice fly, and three dipterous larvæ.
No. 3. A small adult dipterous insect.
No. 4. Unidentifiable animal fragments.
No. 5. Insect larva, unidentified.
No. 6. Spider, probably an *Azalena*, with egg mass.
No. 7. Large black ant.
No. 8. Fragments of beetles, hymenopterous insects, and sowbugs.

No. 9. Two small beetles, a dipterous insect, a small green caterpillar, and an insect larva.

No. 10. Remains of an aquatic insect larva.

No. 11. Sand, dirt, and unrecognizable debris.

No. 12. A small annelid, fragments of a larger annelid, two small crustaceans (probably *Gammarus*), some mites, and fragments of adult insects.

No. 13. An adult dipterous insect, fragments of other small insects, some very minute mites, and unrecognizable debris.

No. 14. A small mite.

No. 15. A whole *Desmognathus* larva.

No. 16. A whole *Desmognathus* larva.

No. 17. Moulted skin of *Desmognathus*.

No. 18. An earthworm, a small spider, and some six or eight specimens of *Gammarus*.

The breathing habits of adult *Desmognathus* deserve especial discussion because of the lungless condition of the animal. As in all amphibians the skin is a most efficient breathing organ, and so long as it is kept in the normal moist condition which the burrowing habit of the species insures, it probably furnishes ample means for the aeration of the blood which circulates through its capillary network. As is usual with amphibians also, the bucco-pharyngeal cavity is made use of to supplement the cutaneous respiration. Bucco-pharyngeal respiration is accomplished by a rapid fluctuation of the floor of the tightly closed mouth, which results in a rapid succession of movements of air in and out through the wide-opened nares. Correlated probably with the lungless condition, is the fact that these fluctuations are far more rapid in *Desmognathus* than in the lunged forms; moreover, *Desmognathus* in common with other lungless forms (*Spelerpes* and *Plethodon* for example) possesses a relatively longer œsophagus provided with muscles by means of which it may be held distended, thus increasing the size of the respiratory surface by the addition of a mucous membrane which was shown by the researches of H. H. Wilder ('01) and Seelye ('06) to be supplied with a rich capillary network, rendering it an efficient breathing organ.

As I have shown elsewhere (Whipple, '06), the lungless salamanders do not have the habit, otherwise common to amphibians, of changing from aerial to aquatic bucco-pharyngeal respiration when in the water, even though they may be kept completely immersed for several days. Under these conditions the external nares are kept tightly closed and the floor of the mouth forcibly drawn in. The immersed condition is of course an unnatural one for *Desmognathus* as is shown by its frantic efforts to escape from the water, but it shows nevertheless no physical ill effects of a prolonged imprisonment beneath the surface, a proof that the skin is under these conditions a perfectly adequate breathing organ.

In his paper on *Desmognathus fusca* and *Spelerpes bilineatus*, H. H. Wilder ('99) pointed out the great possibilities which are presented by both *Desmognathus* and *Spelerpes* as objects for laboratory study. The abundance and widespread occurrence of the two genera, the ease with which they may be collected in both the larval and the adult states throughout the entire college year, and the convenience with which they may be kept alive in the laboratory for months, with practically no care, render the material always available and inexpensive. The following account taken from Wilder ('99) gives valuable suggestions as to methods of keeping such material in the laboratory:

"*Method of Rearing in Confinement.*—The adults of both species, because of their peculiarities in respiration and the consequent necessity of keeping their skin moist, cannot be kept either in water or in a dry atmosphere, but may easily be kept for months or years in an ordinary fernery where the atmosphere is constantly saturated with moisture. I have in my laboratory a large fernery or terrarium, about 2 × 3 feet square and 2 feet high. The bottom consists of a zinc tray, 8 inches deep and water-tight. The top and sides are of glass and the front side runs in a frame with weights, being thus capable of being raised and lowered like an ordinary window-sash. In the bottom of this there are about 6 inches of good garden soil, in which are planted ferns and other wood plants. The surface is partly covered with moss, and here and there are placed several stones, the size of one's fist, and a few pieces of rotten stump, arranged so as to give shelter to the adults. In one corner a crystallizing dish is sunk to the level of the soil. This is filled with water and the bottom covered with a little fine sand. Some duckweed, or *Salvinia*, may be placed upon the surface, and a few small stones should be put in a dish. At the beginning of the season, after arranging everything as above, enough water is poured in to drench the soil, and the sunken dish is filled. After this the terrarium is self-regulating. The water that evaporates is re-precipitated as moisture, and the total loss from the little pond in the corner is so slight that it needs replenishing not oftener than once in six months. If the terrarium is to support many animals, it is better to place a few earthworms, myriapods, etc., in it; and if the pond is designed for the rearing of larvæ, supplies of *Entomostraca* and a little *Spirogyra* to feed them with should be occasionally introduced. I have tried placing tiny bits of meat in prominent places, but they merely mould and have to be removed. I have kept as many as 20–30 adults and a dozen larvæ in my terrarium during an entire college year, and several times, on clearing it out in the fall after the summer vacation, I have found alive and in good condition adults which I had been unable to find in the spring, when I intend always to remove the animals. It seems most probable that these salamanders find enough to eat among the worms and insects introduced with the earth and plants, as they always appear in perfectly normal condition and contrast very forcibly with *Diemyctylus*, which grows thin and often starves to death when placed under the same conditions."

I might add to this account the fact that I have found very convenient for the accommodation of a small number of specimens, such terraria as may be readily constructed from cylindrical anatomical jars of heavy glass, measuring from 8 to 18 inches in diameter and from 6 to 10 inches high, and provided with a tightly fitting but easily removable glass cover. Such jars, equipped with earth, stones, and plants as above directed by Wilder, will accommodate according to size from two to ten specimens of adult *Desmognathus* and are small enough to be easily moved about. Moreover, owing to the fact that the adult *Desmognathus* occasionally feeds upon the larvæ, I have found it safer for the latter to be kept in separate shallow receptacles, such as glass crystallizing dishes, equipped with a little earth covered with water to the depth of a few centimeters, and containing a quantity of decaying, slime-covered leaves which have been transported to it from the natural habitat of the larvæ. Such an arrangement, if kept tightly covered to prevent evaporation, will support the larvæ in a normal condition for months without further care.

The usefulness of *Desmognathus* as material for the various phases of introductory research work cannot be over-emphasized. As Wilder has pointed out, the large size and the unpigmented nature of the egg render it excellent material for the study of general amphibian embryology. The small size of the larvæ (from 15 to 30 mm. in length), and their long continuance in the larval state, make them particularly valuable material for the introduction to methods of study by serial sections, since the specimens are not only small enought to decalcify easily and section beautifully but are large enough and enough like the adult in their general anatomy to also lend themselves readily to study by dissection, and by the numerous methods of injecting and selective staining followed by clearing *in toto*. Studied in this way it furnishes, pedagogically, an excellent means of transition from the study of the gross anatomy of larger vertebrate forms to the study both of their histology and their earlier embryology. Thus it bridges a gap which it has often been customary in college courses to make at a jump which too often lands even the able student in a region of mystery. As material for the study of adult anatomy, also, *Desmognathus* is a convenient form, the larger specimens being quite large enough for ready dissection, and not too large to use with the dissecting microscope for finer details, while both the large and the small adults may be readily decalcified and sectioned for the microscopic study of their structure. Moreover the species with its succession of terrestrial and aquatic stages, its lungless condition and other peculiarities of structure, presents a number of little physiological and anatomical problems, the working out of which is not too difficult for the inexperienced beginner, and may even yield important contributions to our general knowledge.

It is with the hope, therefore, that these pages will be useful and suggestive to those interested in any amphibian problems upon which the study of *Desmognathus* might have some bearing, that it has seemed worth while to collate the known facts concerning the life history of this common and interesting species.

Smith College, Northampton, Mass.,
June 26, 1912.

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List of Abbreviations Used in the Plates.

ac, acinous glands.
cap, capillaries.
cl, columnar epidermal cells.
cor.d, dense corium.
cor.l, loose corium.
cu, cuticle formed by the outer borders of the outer layer of epidermal cells.
d, duct of acinous gland.
ep, epidermis.
ext, outer layer of epidermal cells.

gc, giant cells of the acinous glands.

int, inner layer of epidermal cells.

intr, intrusive cells (Schaltzelle), which project from the deeper layer into the outer layer.

lc, Leydig cells.

leu, leukocytes.

lu, lumen of gland.

ml, moult layer.

mt, mitosis.

mt(*inv*), mitosis in a cell which has invaded a Leydig cell.

pg, pigment.

pgbr, branches of pigment cells.

pgc, pigment cells.

pgc(*inv*), pigment cells which have invaded the Leydig cells.

pgg, intracellular pigment granules.

sn, integumental sense organ.

sp, spireme.

yg, yolk granules.

Description of Plates.

Plates I., II., and III. A series of outlines drawn with Abbé camera, magnification × 4.25, showing various stages of *Desmognathus fusca* from the time of hatching to the attainment of practically adult proportions.

Plates IV., V., and VI. Sections of the skin of *Desmognathus fusca*, showing the structures characteristic of the various periods and the developmental changes which take place in the transition from one stage to another. Drawn with Abbé camera. × 450.

Errata.

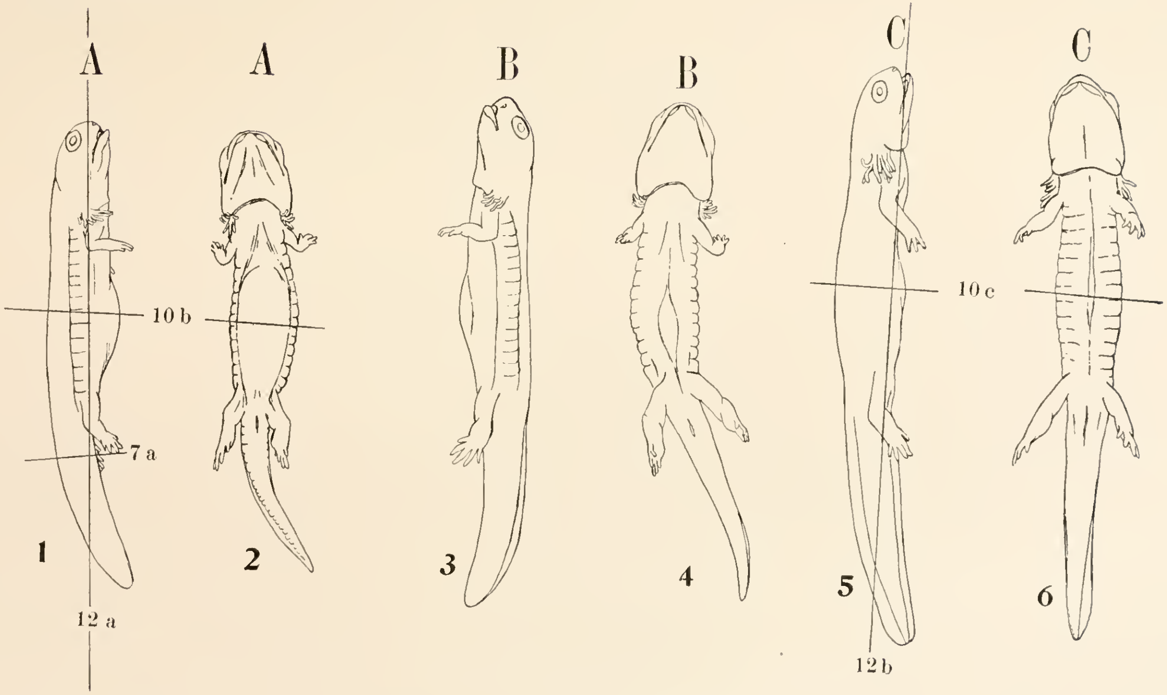
References throughout the text to Pl. I., Fig. 7–11, apply to Pl. II.

Plate I.

Figs. 1 and 2. Lateral and ventral views of terrestrial larval stage *A*, immediately after hatching. Lines *7a*, *10b*, and *12a* show the locations of the sections shown in the text figures correspondingly numbered.

Figs. 3 and 4. Lateral and ventral views of terrestrial larval stage *B*, age 3 days.

Figs. 5 and 6. Lateral and ventral views of terrestrial larval stage *C*, age 3 days. Lines *10c* and *12b* show the locations of the sections shown in the text figures correspondingly numbered.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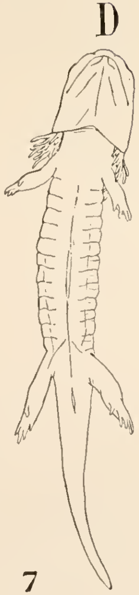
I. W. WILDER.

PLATE II.

FIGS. 7 and 8. Lateral and ventral views of terrestrial larval stage *D*, age 7½ days.

FIG. 9. Lateral view of terrestrial larval stage *F*, a small specimen which has attained the proportions of the aquatic larvæ, age 15½ days. Line *10d* and *12c* show the location of the sections shown in the text figures correspondingly numbered.

FIGS. 10 and 11. Lateral and ventral views of an aquatic larva collected in October. Line *7b* indicates the location of the section shown in the text figure correspondingly numbered. The dotted lines show the levels used in arbitrarily dividing the body into head, trunk, and tail regions for measurements (cf. Tables I., and II., and Graphs I., II., III., and IV.).



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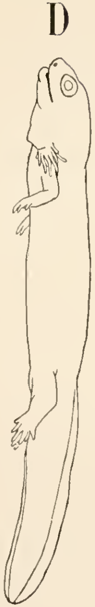

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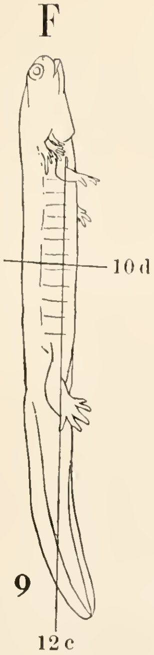

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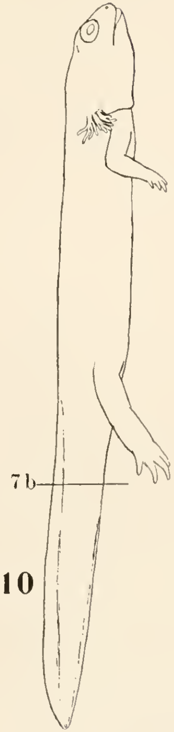

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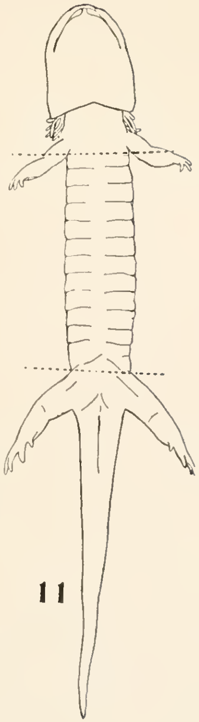

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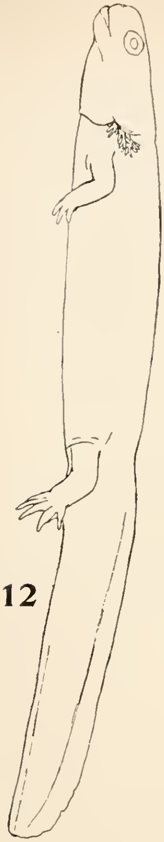
PLATE III.

FIGS. 12 and 13. Lateral and dorsal views of an aquatic larva collected in May, and hence nearly ready to undergo metamorphosis.

FIGS. 14 and 15. Lateral and dorsal views of a small, recently metamorphosed adult, collected in June.

FIG. 16. Ventral view of an adult 49.4 mm. long.



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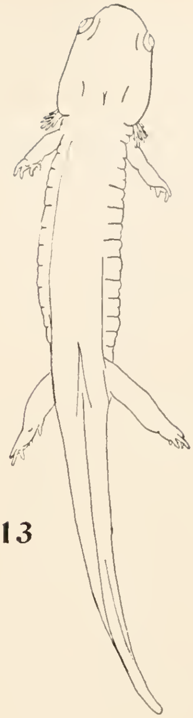

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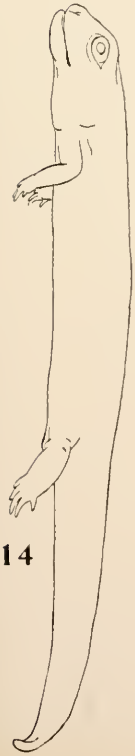

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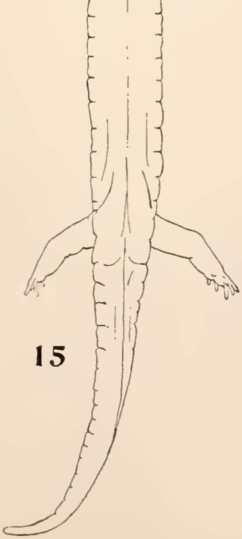

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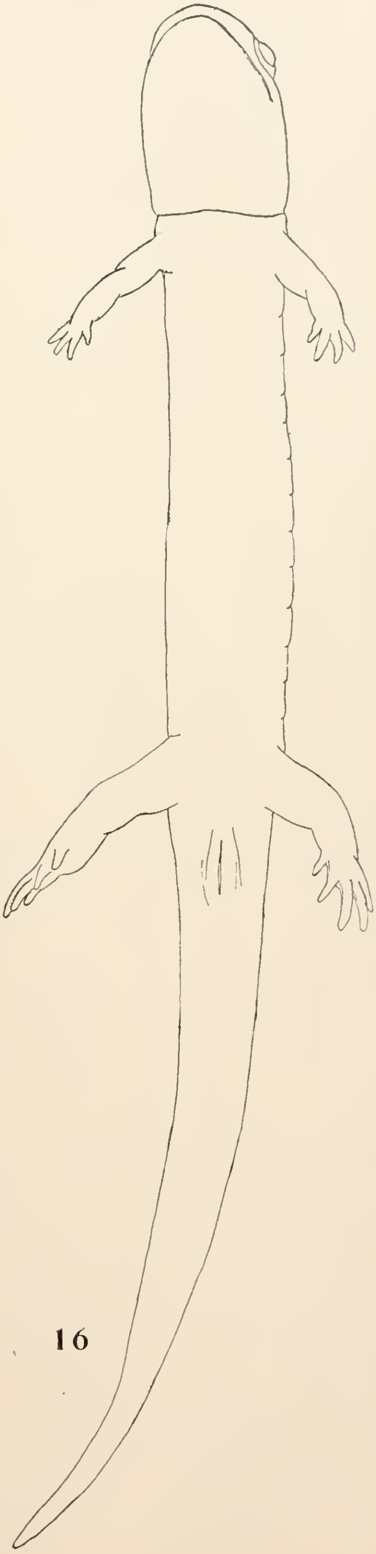

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PLATE IV.

FIG. 17. Vertical section through the skin of the dorsal surface of a 13 mm. embryo of 30 days' development, showing the characteristic two layers of epidermal cells.

FIG. 18. Vertical section through the skin of the lateral surface of a 13 mm. embryo.

FIG. 19. Vertical section through the skin of the dorsal surface of a newly hatched terrestrial larva, stage *A*. Note the presence of a corium of dense connective tissue beneath the two layers of epidermal cells, the absence of mitosis in the epidermis, and the development of an external cuticle from the outer borders of the outer layer of cells.

FIG. 20. Vertical section through one of the integumental sense organs in the region of the gular fold, terrestrial larva, stage *A*.

FIG. 21. Section parallel with the external surface, through the outer layer of epidermal cells, dorso-lateral surface, terrestrial larva, stage *A*.

FIG. 22. Similar section through the inner layer of epidermal cells, dorso-lateral surface, terrestrial larva, stage *A*.

FIG. 23. Vertical section through the skin of the dorso-lateral surface of an aquatic larva collected in September. Note the presence of numerous Leydig cells in the deeper layer.

FIG. 24. Section parallel with the external surface, through the outer layer of epidermal cells from a lateral, unpigmented region of the body of an aquatic larva collected in September.

FIG. 25. Similar section through the inner layer of epidermal cells, lateral region, of an aquatic larva collected in September.

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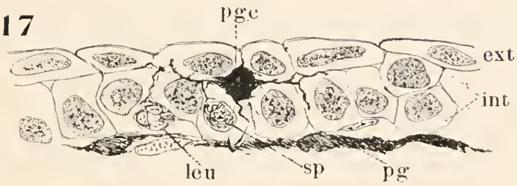
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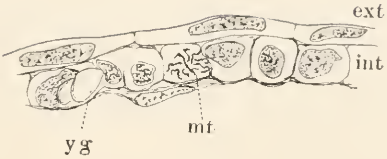
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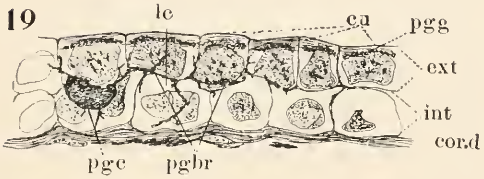
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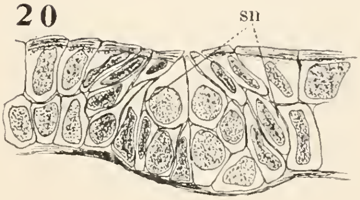

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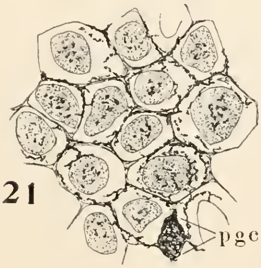

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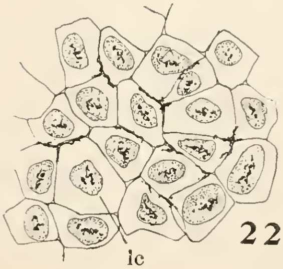

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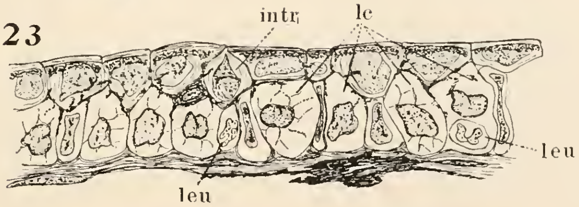
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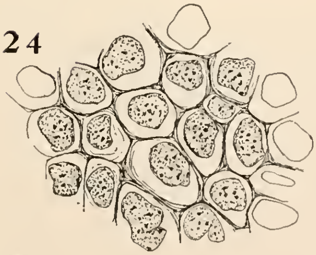

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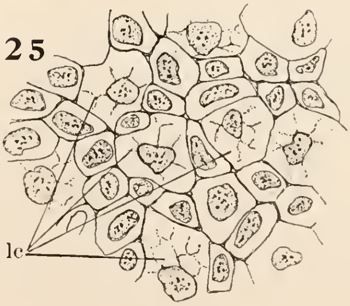


PLATE V.

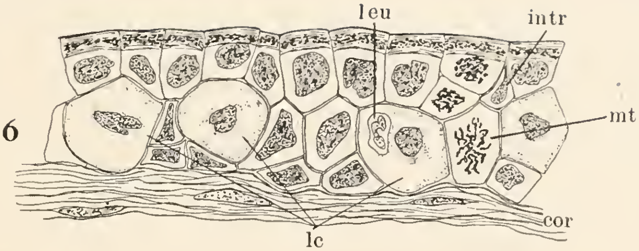
FIG. 26 Vertical section through the skin of the lateral region of the body of an aquatic larva collected in May and hence in preparation for metamorphosis as is shown by the presence of mitotic figures, and by the invasion of leukocytes.

FIG. 27. Section parallel with the external surface, through the outer layer of epidermal cells of the skin of an aquatic larva collected in May.

FIG. 28. Similar section through the inner layer of epidermal cells, aquatic larva collected in May.

FIG. 29. Vertical section through the skin of the ventral surface of the leg of an aquatic larva collected in May and showing the rapid progress of metamorphic changes, among them the increase in the number of cells by mitotic division, the development of adult acinous glands, the invasion of leukocytes, the atrophy of the Leydig cells, and the formation of the loose corium.

FIG. 30. Vertical section through the skin of a larva collected in June in which the metamorphic changes in the skin are nearly completed. Note, in addition to the features indicated in Fig. 29, the presence of a capillary network in the loose corium surrounding the acinous gland.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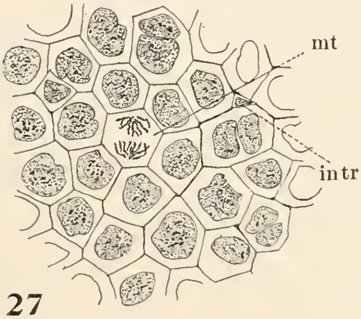
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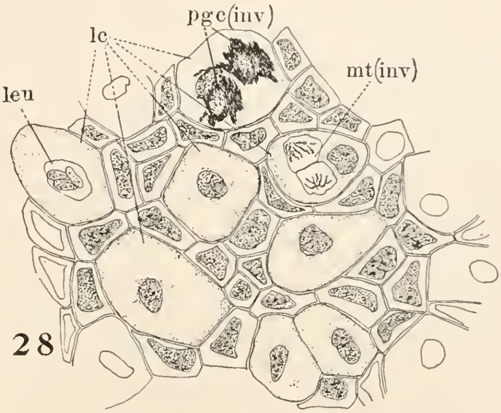
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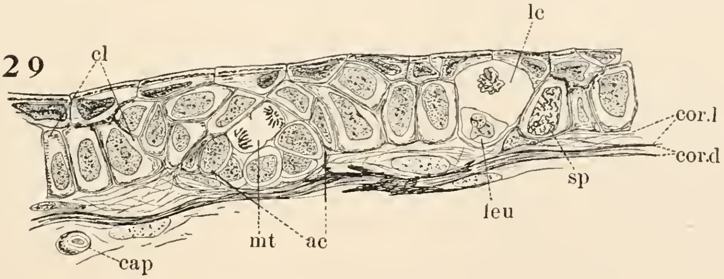
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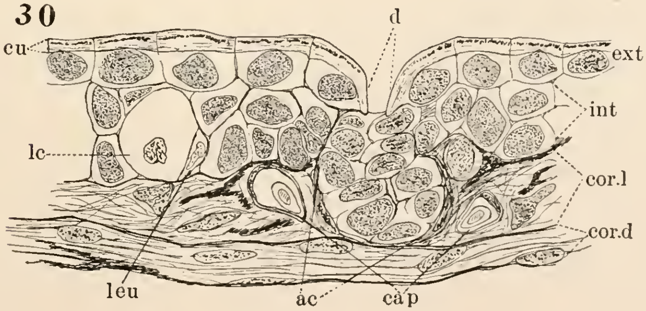
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PLATE VI.

FIG. 31. Vertical section through the skin of an individual which has just completed its metamorphosis, but in which the first moult has not yet occurred. Note the presence of a deeper layer of flattened cells which will become the next moult layer.

FIG. 32. Vertical section through the skin of a very small adult (length 29 mm.) collected in June shortly after metamorphosis and after the first moult has been cast.

FIG. 33. Vertical section through the skin of a large adult, showing the large size of the acinous glands, the great thickness of the loose corium, and the relative thinness of the epidermic layer (cf. larval condition).

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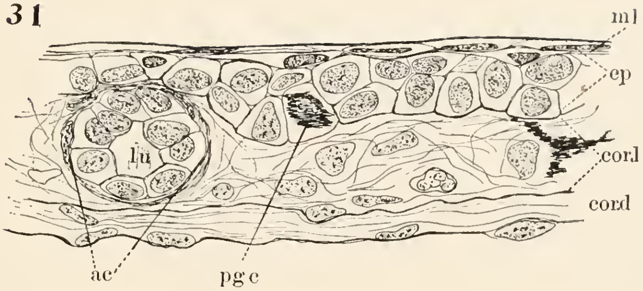
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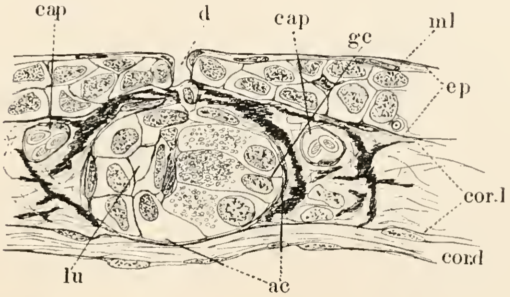
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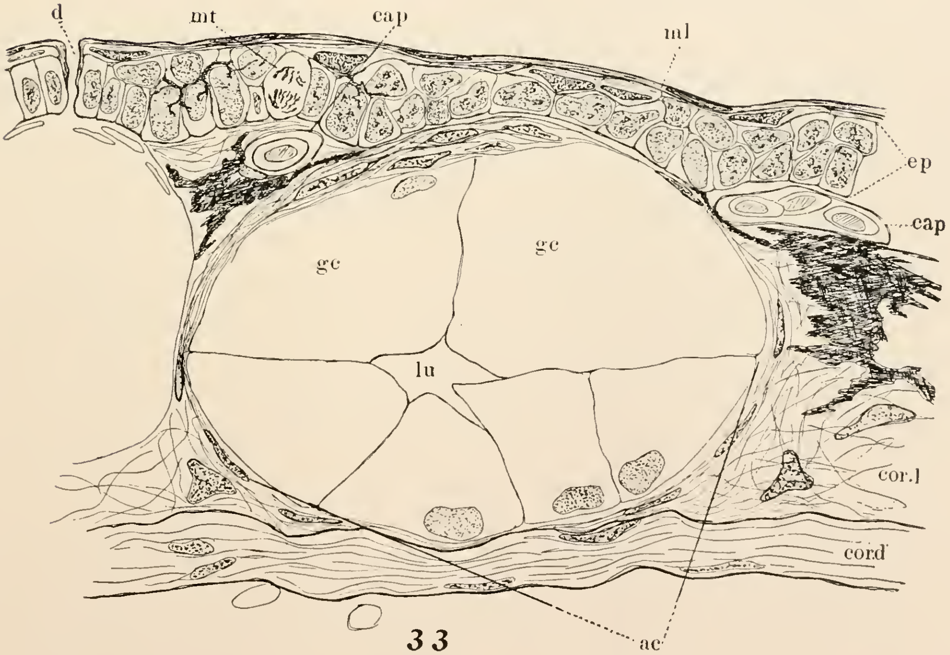
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33

STUDIES IN ARTIFICIAL PARTHENOGENESIS.[1]

I. Membrane Elevation in the Sea-Urchin Egg.

LEWIS V. HEILBRUNN.

Introduction.—The ultimate goal in the study of artificial parthenogenesis is the discovery of the chemical and physical forces which are assumed to cause the initiation of development. The pioneer workers on the problem (Tichomiroff, O. and R. Hertwig, Morgan) were content to regard the phenomenon as the result of a stimulus which arouses the egg from its resting condition. But the present tendency, thanks to the work of Loeb and his school, recognizes that such a theory is merely a cloak for our ignorance, it is little more than a restatement of the problem. For it gives us no information as to the real nature of the assumed stimulus, or in what manner it effects its action. The foremost difficulty in the way of a physical or chemical interpretation is the wide range of chemicals and forces all of which are capable of producing artificial parthenogenesis. None of the theories recently advanced seems capable of explaining all or nearly all of the facts. As a result there has been of late again a return to those vague ideas which challenge attack.

In almost all hypotheses of artificial parthenogenesis, the process, as a result of which a membrane is either pushed out or formed "de novo" around the egg, plays an important rôle. This so-called "membrane formation" is readily observed in the egg of the sea-urchin, and it is in *Arbacia* that I have attempted to study it. I am indebted to Prof. T. H. Morgan for the use of a Columbia table in the Marine Biological Laboratory at Woods Hole, Mass., where I spent the summer of 1912.

The form studied was *Arbacia punctulata* (Gray). The nomenclature of the membranes of the sea-urchin egg is somewhat varied, and it will be necessary to decide on a series of terms, which, for the sake of clearness, shall be exclusively used. As it emerges from the ovary the unfertilized egg is already surrounded by a broad band of gelatinous material, the chorion. Directly underneath the chorion lies the peripheral surface of the egg. After fertilization, a membrane is clearly seen beneath the chorion and some little distance from the egg; this is the vitelline (or so-called fertilization membrane). The presence of this membrane in the unfertilized egg has often been disputed. Hertwig[1] was of the opinion that a definite preformed membrane exists. Such a condition seemed to Fol[2] to preclude the penetration of the sperm, a fact which he had for the first time observed. Accordingly, he states that the egg is not surrounded by a membrane, but by a "hyaline layer." Upon fertilization, he observes that this layer becomes lifted from the egg and forms the vitelline membrane. Herbst[3] first showed that this "hyaline layer" of Fol was a true membrane in that it possessed rigidity and was distinct from the underlying cytoplasm. He found on pressing unfertilized eggs under a cover glass, that the protoplasm of the egg flowed out, leaving behind the membrane.[4] Schücking[5] isolated the membrane by cutting the eggs. Finally Kite[6] has been able to dissect away the membrane with the aid of a Barber pipette. No doubt the difficulty in observing the membrane is due to the fact that its refractive index is almost identical with that of sea-water. If the refractive index of sea-water is increased, *e. g.*, by the addition of a protein, the vitelline membrane becomes clearly visible. The observation of this membrane in such liquids of greater refractive index has led several observers to suppose that it is "formed" there. Since membranes "formed" in this way are penetrable to sperm, Loeb[7] has proposed to call them "pseudomembranes."

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[1] From the Cornell University Laboratory of Embryology.

In as much as the presence of a preformed membrane has been demonstrated by a number of observers, it is incorrect to speak of the "formation" of a vitelline membrane at fertilization.

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[1] O. Hertwig, *Morph. Jahrb.*, I., 347 (1875).

[2] H. Fol, *Ann. d. sci. phys. et nat.*, LVIII., 439 (1877); *ib.*, *Mem. d. l. Soc. d. phys. et d'hist. nat. d. Genève*, XXVI. (1879).

[3] C. Herbst, *Biol. Centralbl.*, XIII., 14 (1893).

[4] This fact had already been noted by Hertwig (*loc. cit.*).

[5] Schücking, *Arch. f. d. ges. Physiol.*, XCVII., 68 (1903).

[6] G. L. Kite, *Science*, N. S., XXXVI., 562 (1912).

[7] J. Loeb, *Arch. f. Ent. Mech.*, XXVI., 82 (1908).

Thus, although this phrase is in general use, I have discarded it in preference for the more truly descriptive term "elevation." This is in accord with the German practice.

Loeb claims that the process of normal membrane elevation is too rapid for observation. It is necessary to retard the process by cooling to 3° or 4°, when, on fertilization under these conditions, small rounded elevations, the so-called "bubbles" appear on the egg surface; these flow together and their fused outer boundary becomes the vitelline membrane. A similar effect is observed on treatment with butyric acid. I believe that bubble formation is abnormal, and I shall consider its true cause later.

In the fertilized egg the space between the vitelline membrane and the egg is known as the perivitelline space (Membranraum). Some few minutes after fertilization a hyaline layer appears to be differentiated at the external border of the cytoplasm. This is the hyaline layer (ectoplasmic layer, etc.).

The importance of a study of membrane elevation is twofold. In the first place, it may serve as a device for the prevention of polyspermy in normal fertilization, and is, therefore, of physiologic significance. Secondly, in recent years, this process has played an important part in theories of fertilization and artificial parthenogenesis. Robertson[1] goes so far as to confuse membrane elevation with fertilization, considering the two as synonymous.

Although from time to time various ingenious hypotheses have been advanced to explain membrane elevation, the only theory at all plausible was advanced by Fol[2] who was the first ever to consider the problem. Before discussing Fol's view I shall first mention several other explanations which have been suggested. Schücking[3] thought that the preformed membrane was split by the absorption of water, so that a double membrane was produced on fertilization. Such a process would be difficult to explain physically, and no one has ever attempted to do so. Fischer and Ostwald[4] regard membrane elevation as due to the exit from the egg of a fluid which is given off as a result of coagulation of a portion of the protoplasm. The rapidity with

[1] T. B. Robertson, *Arch. f. Entw. Mech.*, XXXV., 64 (1912).

[2] Fol, *Mem. d. l. Soc. d. phys. et d'hist. nat. d. Geneve*, XXVI. (1879).

[3] Loc. cit.

[4] M. Fischer and Wo. Ostwald, *Arch. f. d. ges. Physiol.*, CVI., 229 (1905).

which the membrane is thrust out is but one of the difficulties which such a theory would have to surmount. Traube[1] considers artificial membrane elevation as the result of a secretion after a return to normal sea-water, but in all but a few cases, such a return to sea-water is not necessary. McClendon[2] is of the opinion that an electro-positive (or acid) colloid secretion is[3] poured out from the egg which reacts with the supposedly electro-negative chorion to produce the vitelline membrane. This view is, of course, not in accord with the evidence cited above in favor of the preëxistence of a vitelline membrane. Elder[4] has recently adopted a conclusion identical with that of McClendon, to whom, however, he makes no acknowledgment. He states that the vitelline membrane does not "form" on eggs which lack a chorion. But these eggs are evidently not in good condition, they are probably overripe, and such eggs have been shown by Fol and Loeb incapable of pushing out a vitelline membrane. Elder also states that if the chorion be removed, membrane elevation does not occur (a fact previously claimed by McClendon) but his method of removal involves keeping the eggs 12–14 hours in artificial sea-water. This, of course, precludes membrane elevation. Such evidence is directly controverted by the fact observed by Lyon[5] that after a most vigorous treatment in the centrifuge normal membrane elevation occurs. In eggs treated in this manner the chorion is always absent.

Fol[6] in discussing membrane elevation in *Asterias* eggs, a process which he states is exactly similar to membrane elevation in the eggs of the sea-urchins, offers the following explanation. A jelly exists between the surface of the egg and the elevated membrane, for the distance between the egg and membrane always remains uniform on all sides. The pushing out of the membrane is due to the absorption of water by this gel. Herbst[1] in a study of membrane elevation in sea-urchin eggs, supports Fol's interpretation. A similar view is likewise expressed by Krassuskaja and Landau[2] and by R. Hertwig.[3] Nevertheless, the view is sometimes regarded as due to Loeb, who first proposed it in 1908. Let us follow the latter's reasoning a little more closely.

---

[1] J. Traube, *Biochem. Zeitsch.*, XVI., 182 (1909).

[2] J. F. McClendon, BIOL. BULL. XXII., 157 (1912).

[3] As proof that this colloid, which is assumed to lie directly beneath the membrane after fertilization, is electro-positive, McClendon states that the passage of an electric current through fertilized eggs in sea-water causes the membrane to "bulge out" towards the cathode. This effect, however, may very well be due to an attempted migration of the egg in the direction of the anode, towards which electrode, most if not all cells migrate (probably because of the alkalinity of protoplasm). The fact that eggs which have ruptured their membranes do not migrate to the anode (McClendon, *Amer. Journ. Physiol.*, XXVII., 273 (1910)) is probably due to a retarding action of the film of water adjoining the glass slide (see W. B. Hardy and H. W. Harvey, *Proc. Roy. Soc.*, B. LXXXIV., 217 (1911)).

[4] J. C. Elder, *Arch. f. Ent.-Mech.*, XXXV., 145 (1912).

[5] E. P. Lyon, *Arch. f. Ent. Mech.*, XXIII., 151 (1907).

[6] Loc. cit.

In 1900, he regarded membrane elevation as due to coagulation. In 1905,[4] he thought of it as a secretion. In 1907 he began to consider it as due to liquefaction. He first proves that in general the same types of treatment will produce hæmolysis of the red blood cells and the throwing off of a membrane in *Arbacia*. Then he proceeds to adopt the current theory of hæmolytic action to account for the membrane elevation. Köppe and others had attempted to show that the solution or liquefaction of lipoids was necessary for hæmolysis. Accordingly, Loeb[5] expresses the view that membrane elevation is primarily due to a solution of lipoids at the periphery. This view was also taken up in Loeb's laboratory by v. Knaffl-Lenz,[6] who brought forth evidence that the lipoid in question was lecithin. But the actual force which produced the membrane elevation Loeb believes to be due to the swelling of a colloid. He conceives of this colloid as being given off at the time of fertilization and immediately swelling and thus distending the membrane. This does not accord with his previous statements that the unfertilized egg is naked, but Loeb surmounts this difficulty by assuming an "Oberflächenlamelle" or a differentiated surface layer. In 1909, Loeb[7] is no longer certain that solution of lipoids is necessary, but he still clings to the view that the swelling of a colloid is involved. This is the identical view earlier expressed by Fol, Herbst, etc. Because of the similarity between membrane elevation and hæmolysis, Loeb introduces the term lysine to denote any substance of unknown chemical composition which will produce membrane elevation and cytolysis. It is evident that the sperm may contain a lysine.

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[1] C. Herbst, loc. cit.

[2] Krassuskaja and Landau, *Biol. Centralbl.*, XXIII., 613 (1903).

[3] R. Hertwig in O. Hertwig's *Handbuch der Entwicklungslehre der Wirbeltiere*, I. (1), p. 484.

[4] Univ. Cal. Publ. Physiology, II., 123 (1905).

[5] Loeb, *Arch. f. d. ges. Physiol.*, CXX., 196 (1908).

[6] v. Knaffl-Lenz, *Arch. f. d. ges. Physiol.*, CXXIII., 279 (1908).

[7] Loeb, "Chem. Entwicklungserregung," p. 248.

R. S. Lillie[1] suggests a possible cause of swelling. He assumes that at fertilization the outer surface layer of the egg becomes more permeable to the salts of sea-water. These can now exert no osmotic pressure against it, but since this outer film or membrane must still remain impermeable to the colloids within, these do exert osmotic pressure,[2] and an inflow of water with consequent swelling of some of the peripheral cytoplasm occurs. The cause of such an increase in permeability has only been hinted at. Its actual occurrence Lillie bases on the evidence of McClendon, Lyon and Shackell, and Harvey. But even granted such a change of permeability, might it not just as well be regarded as a result rather than as a cause of membrane elevation? Harvey's[4] view is very much the same. He agrees with Loeb that the membrane elevation is due to the swelling of a colloid, and at the same time brings evidence in favor of Lillie's hypothesis of increased permeability of plasma membrane. But Harvey also believes that the presence of the "membrane substance" is due to a reaction or reactions brought about by the escape of CO_2 as a result of increased permeability. Unfortunately, however, he does not bring up any evidence in favor of this accessory hypothesis, and it is, indeed, difficult to understand how both reaction and swelling can take place in so short a time.

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[1] R. S. Lillie, *Amer. Journ. Physiol.*, XXVII., 301 (1911).

[2] Lillie considers osmotic pressure and "Quellungsdruck" synonymous, but this is obviously improper, for the osmotic pressure of colloids is almost negligible, the "Quellungsdruck," on the other hand, may exert a pressure of over 40 atmospheres.

[3] J. F. McClendon, *Amer. Journ. Physiol.*, XXVII., 240 (1910); Lyon and Shackell, *Science*, N. S., XXXII., 249 (1910); Harvey, *Science*, N. S., XXXII., 565 (1910). The first two papers do not prove increased permeability of the membrane. They only show that fertilized eggs stain more readily than those not fertilized, a phenomenon which may also depend upon an increased rate of adsorption after fertilization, or to a lowering of osmotic pressure, such as Bachmann (*Arch. f. d. ges. Physiol.*, CXLVIII., 141 (1912)) concludes for the egg of *Triton*. McClendon's results are also unsatisfactory. He observed an increased conductivity after fertilization, and from this concluded an increased permeability of the outer layer or membrane. Such an increase in conductivity may well have been caused by a disintegration of some of the eggs. The action of the electric current, or the centrifuging which preceded conductivity determinations, might readily produce disintegration.

[4] E. N. Harvey, *Journ. Exp. Zoöl.*, VIII., 355 (1910).

The appended list is an enumeration of all the various methods by which membrane elevation may be artificially induced in sea-urchin eggs. Sometimes artificial parthenogenesis has been described without any records to show if membrane elevation occurred. Several of these methods are included in the list, where they are followed by a question mark. Most acids only cause membrane elevation after the eggs have been restored to normal sea-water, but in all other cases the process occurs directly.

- So-called lipoid solvents
  - Chloroform (Hertwig).
  - Toluol, benzol, xylol, oil of cloves, creosote (Herbst).
  - Ether, alcohol (Matthews).
  - Amylene, phenol (Loeb).
- Distilled water (Schücking).
- Dilution of sea-water (Schücking).
- Isotonic NaCl, KCl (Lillie).
- Soap (Loeb).
- Saponin, digitalin, solanin (Loeb).
- Bile salts (v. Knaffl-Lenz).
- Sea-water charged with CO_2 (Delage, Lyon).
- Passage of hydrogen and oxygen (Matthews).
- Shaking (?) (McClendon).
- Heat (34° Loeb) (32° (?) McClendon).
- Alkalis (Loeb, Schücking).
- KCN (?).
- Metallic copper or silver (Herbst).
- Electric current (?) (Schücking).
- Blood serum (Loeb).
- Oöcytin (Robertson).
- Higher fatty acids (Loeb).
- Lower fatty acids (Loeb),
- Hydroxi-acids (Loeb),
- HNO_3 and HCl (Loeb),
  - Membrane elevation only after return to sea-water.

I shall now attempt to prove that every known method of producing membrane elevation results in a lowering of the surface tension of the liquid surrounding the egg. Proceeding in order, the first class of substances noted are those often grouped as lipoid solvents. The surface tension of some of these substances is listed in Landolt and Börnstein, "Physicalische Chemische Tabellen."

Substance.	Temperature.	Surface Tension (Dynes per Cm.)
Chloroform	20°	26.72
Toluol	17.5°	28.52
Benzol	20°	30.2
Xylol	15.7°	28.97
Ether	20°	16.49
Alcohol	20°	22.03
Amylene[1]	16.5°	17.21
Phenol	36.5°	41.3

It will be noticed that the first four of these substances are practically insoluble in sea-water. Herbst found that chloroform and toluol must be shaken with sea-water to produce the necessary effect.

Distilled Water and Dilution of Sea-water.—The surface tension of distilled water is 75 dynes per centimeter. The addition of inorganic salts always increases the surface tension gradually in proportion to the concentration of each salt present, and, by the work of Valson, Röntgen and Schneider, Whatmough,[2] etc., we should estimate the surface tension of sea-water at approximately 77 dynes per centimeter. Hence diluted sea-water and distilled water have a somewhat lower surface tension than sea-water, and it is to this fact that I attribute their action in causing membrane elevation.

Isotonic Salt Solutions.—Lillie[3] first showed that pure isotonic (*i. e.*, 55M) solutions of sodium salts caused membrane elevation, the order of effectiveness of anions being that of the lyotropic series, $Cl > Br > ClO_3 > NO_3 > CNS > I$. The effect of these salts was inhibited by $CaCl_2$ and $MgCl_2$. It was shown by Whatmough[4] and others that equivalent normal solutions of chlorides raise the surface tension of water by approximately equal amounts. Hence a solution of a bivalent salt (such as $MgCl_2$ or $CaCl_2$), which is equal in molecular concentration to a solution of NaCl, gives an increase of surface tension which is about double that produced by the NaCl. Sea-water, consisting as it does of monovalent and bivalent salts, has a higher surface tension than an isotonic solution of sodium chloride (55M). It also contains sulphates which raise surface tension more than monovalent chlorides. The fact, therefore, that isotonic solutions of NaCl, etc., do cause membrane elevation is exactly in accord with my view. Moreover, the addition of $CaCl_2$ and $MgCl_2$, two bivalent salts, raises the surface tension, and, hence, as might be expected, membrane elevation no longer takes place. Finally, the additional evidence of the correctness of my interpretation is furnished by the order of effectiveness of the ions: $Cl < Br < ClO_3 < NO_3 < CNS < I$. For we know the surface tension increasing powers of the anions to be $Cl > Br > NO_3 > I$.[1]

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[1] The surface tension of amylene is not given in Landolt and Börnstein, so I have taken the value given in Castell-Evans' Physico-Chemical Tables. Creosote is not included in the list as it is a mixture of various substances; most of these, however, have a lower surface tension than water.

[2] Valson, *Ann. de chem. et de phys.* (4), XX., 361 (1870); Röntgen and Schneider, *Wied. Ann.*, XXIX., 165 (1886); Whatmough, *Zeit. f. phys. Chem.*, XXXIX., 129 (1902).

[3] R. S. Lillie, *Amer. Journ. Physiol.*, XXVI., 106 (1910).

[4] *Loc. cit.*

Soap.—Na oleate greatly lowers the surface tension of water[2] and Loeb[3] finds that when it is added to M/2 NaCl it increases the membrane elevating power of the solution. The fact that as much as 2 per cent. of Na oleate is necessary is probably due to the tendency of the NaCl present to salt it out of solution.

Saponin.—Freundlich[4] determines the surface tension of a solution of saponin as 52 dynes per cm. Probably solanin and digitalin also lower surface tension.

Bile Salts.—These substances are extremely effective in lowering surface tension. For example, Lewis[5] gives the surface tension of a 0.2 per cent. solution of sodium glycocholate as 44.98 dynes per centimeter (at 14°).

Sea-water Charged with CO_2.—The surface tension of gases is zero, hence we should expect them to lower the surface tension of water when dissolved in it. This assumption was proven correct by Bellati and Lusanna[1] and more recently by Bönicke,[2] who finds that ten volumes per cent. of CO_2 lower the surface tension of water by about one dyne.

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[1] Röntgen and Schneider, *Annalen der Physik u. Chem.*, XXIX., 209 (1886); Traube, *Journ. Prakt. Chem.*, CXXXIX., 177 (1885).

[2] Donnan, *Zeits. f. phys. Chem.*, XXXI., 42 (1899).

[3] Loeb, "Chem. Entwicklungserregung," 1909, p. 140.

[4] "Kapillarchemie," p. 56.

[5] Lewis, *Zeit. f. physik. Chem.*, LXXIV., 619 (1910).

Passage of Hydrogen and Oxygen.—Matthews[3] found that if hydrogen be passed through sea-water containing eggs for ten minutes, then oxygen for ten minutes, and once more hydrogen for ten minutes, the eggs thus treated begin to develop. He does not state whether membrane elevation occurs, but we can readily understand that it may well do so, for both hydrogen and oxygen, no doubt, lower the surface tension.[4] As Matthews says he ran the hydrogen through swiftly, the impurities (*e. g.*, SO_3, AsH_3) could not have been completely removed, and probably, for this reason, continued exposure to hydrogen gas was found injurious.

Shaking.—Matthews[5] finds that shaking will cause artificial parthogenesis in starfish eggs, but met with no success in his experiments in *Arbacia*. However, McClendon reports that segmentation occurred after shaking in a vial for five minutes. He does not state if membrane elevation occurred, but from a chance experiment of my own, I believe that it may do so. Vigorous shaking would produce a lowering of surface tension in that it would increase the amount of air absorbed (for effect of gases see above).

Heat (*to 32° or Over*).—As is well known, a rise of temperature produces a lowering of surface tension in all cases. McClendon states that exposure to 32° for four minutes is sufficient to produce segmentation, but he does not state if membrane elevation occurred.

Alkalis.—Upon the addition of NaOH, KOH, or Na_2CO_3, to sea-water, there is an immediate precipitation of magnesium hydroxide. This fact has never been noted by Loeb, McClendon, or Schücking, all of whom have used alkaline sea-water. It is, however, a necessary consequence of the fact that sea-water contains salts of magnesium (which constitute nearly 1/6 of the total salt content). Magnesium salts are always precipitated in the presence of NaOH or KOH, this reaction often being employed in qualitative analysis. As a result of this precipitation, the salts which tend most to raise the surface tension (see p. 350) are precipitated and the surface tension of the sea-water decreases slightly. As might be expected, alkalis are not very powerful agents in producing membrane elevation.

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[1] Bellati and Lusanna, *Atti. 1st Venet.* (6), VII. (1889); ref. in *Wied. Beibl.*, XIV., 18 (1889).

[2] K. Bönicke, Dissert., Münster (1905).

[3] A. P. Matthews, *Amer. Journ. Physiol.*, IV., 343, 1901.

[4] The latter gas was investigated by Bönicke, who found that it lowered surface tension.

[5] A. P. Matthews, *Amer. Journ. Physiol.*, VI., 142 (1902).

Blood Serum.—Loeb[1] first showed that the body fluid of certain annelids of the family Sipunculidæ was capable of producing membrane elevation even in considerable dilution. As I know nothing of the chemical nature of this fluid, it is useless for me to consider this case. Soon after, he found that mammalian blood serum was also effective.[2] 6.5 volumes of the serum are mixed with one volume of 2.5 M NaCl and the resultant solution is then diluted with 1–9 parts of sea-water. Loeb's results show that the per cent. of eggs which "form a membrane" as a result of treatment with this solution is small, in the majority of cases not a single egg throws off its membrane. In order to obtain a higher percentage of membrane elevation, sensitization must be resorted to. This may be effected either by heating (to 31°–34°) or by the addition to the serum of 3/8 M $BaCl_2$ or $SrCl_2$.

First I shall consider the effects of sensitization, as I believe that this process is in itself capable of producing membrane elevation. The result of heating has already been considered, it having been noted that a lowering of surface tension results. As for the addition of $BaCl_2$ or $SrCl_2$, the result must be obvious. Either one of these salts causes an immediate precipitation of sulphates. Loeb in fact mentions a precipitate of $BaSO_4$ in the case of $BaCl_2$, but fails to do so in the case of $SrCl_2$. Such a precipitation has two effects. (1) The strontium chloride added is precipitated from the solution, which then becomes more dilute. (2) The sulphates of sea-water are replaced by chlorides which are somewhat less effective in elevating the surface tension (see p. 351). Thus sensitization is not such a mysterious process as Moore[1] and Robertson[2] would have us believe when they suppose that it is similar to the process of mordanting before dyeing. An experiment of Moore's seems to show that my explanation is correct. Upon placing eggs in 3/8 $SrCl_2$, and then removing directly into sea-water, sensitization occurs, but if the eggs are first washed in NaCl before being placed in sea-water, the effect of the $SrCl_2$ disappears. Evidently the washing away of the $SrCl_2$ prevents its reaction with sea-water.

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[1] J. Loeb, *Arch. f. d. ges. Physiol.*, CXVIII. (1907).

[2] J. Loeb, *Arch. f. d. ges. Physiol.*, CXXII., 196 (1908); CXXIV., 37 (1908).

The effect of serum alone is very slight. Of nine samples of ox-sera tried by Robertson, only one was effective upon sensitized eggs. I attribute the effect of blood serum to two causes. In the first place, before dilution with sea-water the salt content of the "isotonic" serum is almost wholly NaCl, together with a small amount of KCl. The effect of isotonic NaCl and KCl solutions has already been discussed. Secondly, blood serum contains considerable amounts both of oxygen and of carbon dioxide. The total volume of these gases contained in blood varies greatly. On the average, arterial blood possesses about 20 vols. per cent. of oxygen and 40 vols. per cent. of carbon dioxide, whereas venous blood contains approximately 7 volumes per cent. of oxygen and 50 volumes per cent. of carbon dioxide.[3] Blood serum is richer in carbon dioxide than the blood itself.[4] Thus the serum used by Loeb to produce membrane elevation must contain from 5 to 25 volumes per cent. of carbon dioxide in addition to a lesser amount of oxygen.

No doubt some of this CO_2 is loosely combined, but even in this case it would exert a dissociation pressure which would increase the amount present in solution. Using ox-blood obtained from a slaughter-house, Robertson found a difference in the serum obtained from dark and that obtained from light blood, the former being in all cases more effective. He is at a loss for an explanation, but suggests that the dark blood was obtained from animals deprived of water for some time. Probably the dark color of the more effective blood is associated with the presence of a higher per cent. of CO_2. The action of CO_2

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[1] A. R. Moore, Univ. of Cal. Pub'. (Physiology), IV., 91 (1912).

[2] T. B. Robertson, *loc. cit.*, p. 345.

[3] See Oppenheimer's "Handbuch der Biochemie," IV. (1).

[4] Hammersten, "Physiological Chemistry," 6th Amer. edition, p. 804.

(and oxygen) in lowering surface tension has already been discussed.

KCN.—Dilute potassium cyanide is practically equivalent to KOH + HCN, as it undergoes hydrolysis. Hence, the statements in regard to alkalis apply also here.

Metallic Copper or Silver.—Herbst[1] found that membrane elevtion was induced by the presence of metallic Cu or Ag. His results were confirmed by Matthews,[2] whose experiments however were performed on starfish eggs. Herbst used silver reduced from silver nitrate, and also a silver coin. In the former case, silver nitrate is probably present and effects a precipitation of chlorides with a consequent dilution of the sea-water and an exchange of chlorides in solution to nitrates. (For a consideration of the effects of dilution, and of the relative effects of various ions, see p. 350.) In the latter case, it is necessary to note that all silver coins are alloys of copper, a metal which Matthews finds to be much more potent than the silver coin itself.[3] The action of copper is due to the fact that this metal is attacked by NaCl in the presence of air. As a result of this reaction copper oxychloride is formed and the solution becomes alkaline.[4] Thus the action of copper is a special case of the action of alkalis, and the metal does not produce an "action at a distance," as Matthews supposes.

Electric Current.—Although an electric current has been used by Schücking and McClendon, neither of these authors states whether membrane elevation occurs. In both cases, copper wires were probably used, so that membrane elevation may have been induced by the action of copper itself, as shown above.

Oöcytin.—This is an hypothetical substance isolated by T. B. Robertson[5] from blood serum and from sperm. Although called a "fertilizing agent" it produces normal membrane elevation on "sensitized" eggs only. On "unsensitized" eggs it produces agglutination, and sometimes "blister" formation. Its power of producing membrane elevation is, therefore, chiefly due to the process of "sensitization," the real nature of which has already been considered. The agglutinating effect is due to the presence of free hydrochloric acid. Robertson prepares oöcytin by precipitating blood serum with $BaCl_2$ dissolving the precipitate in n/10 HCl, reprecipitating with acetone, and then redissolving in n/10 HCl. The result of this process can not be other than the isolation of a protein combined with HCl, for we know both $BaCl_2$ and acetone as protein precipitants.[1] And, although Robertson exactly neutralizes his final product with NaOH, he, of course, does not neutralize the combined acid which has no action on the color of the indicator. On dilution, the combined acid is split off. Thus Robertson finds that a dilution of one part in two hundred is necessary in order that the oöcytin become effective. Dilute HCl will produce agglutination in the same way as oöcytin.

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[1] C. Herbst, *Mitth. a. d. zool. Station z. Neapel*, XVI., 445 (1904).

[2] A. P. Matthews, *Amer. Journ. Physiol.*, XVIII., 39 (1907).

[3] Metallic silver may itself react for NaCl in the presence of oxygen changes it into AgCl with the simultaneous formation of NaOH. (See Gmelin-Kraut, *Lehrbuch der anorg. Chemie*, II. (2), p. 26.) This reaction probably takes place very slowly, however.

[4] Tilden, *Soc. Chem. Ind.*, V., 84 (1886).

[5] T. B. Robertson, *Arch. f. Ent. Mech.*, XXXV., 64 (1912).

Higher Fatty Acids.—Loeb[2] finds that very dilute solutions of heptoic, octoic, nonoic, and caproic acids produce membrane elevation in eggs exposed to them. As Forch[3] and others have shown, the higher fatty acids lower surface tension markedly, even when present in great dilution. The readiness with which a fatty acid lowers surface tension is found to be in direct relation to the number of carbon atoms it contains.

Lower Fatty Acids. Mineral Acids.—There remain to be considered only those cases in which membrane elevation occurs after a return to sea-water. In all of these cases, the solutions in which the eggs are first placed are acidified. All of the acids used lower surface tension and their effectiveness is in direct measure to the readiness with which they do so. The lower fatty acids, formic, acetic, propionic, butyric, and caproic, lower surface tension markedly in solution, and this power increases as we ascend in the series.[4] Similarly, the effectiveness in producing membrane elevation increases with the number of carbon atoms.

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[1] Mann ("Physiological Histology," p. 102) finds acetone a precipitant of serum globulin and other proteins.

[2] Loeb, "Chemische Entwicklungserregung," 1909, p. 110.

[3] Forch, *Wied. Ann.*, LXVIII., 801 (1898).

[4] Forch, *loc. cit.*

Dibasic acids, such as oxalic, succinic, and tartaric, do not lower surface tension nearly so much[1] and they have comparatively little effect upon sea-urchin eggs. Hydroxi-acids act in a fashion similar to the dibasic acids. Finally, hydrochloric and nitric acids have been found to be least effective.[2] These acids lower the surface tension but slightly, as has been shown, for example, by Röntgen and Schneider.[3] Loeb[4] could obtain no results with sulphuric acid, and it is interesting to note that this acid does not produce a lowering of surface tension when dilute. The question arises as to the reason that a return to sea-water is necessary in the case of the above mentioned acids. This point will be made clear later.

I have now shown that every known method of producing membrane elevation results in a lowering of surface tension. I also found that a number of hitherto untried substances which lower surface tension, can also be used to produce membrane elevation.

Acetone.—According to von Knaffl-Lenz's view that it is the liquefaction of lecithin which causes membrane elevation (see p. 8, l. 12), acetone should be entirely ineffective, as it is well known for its power of precipitating lecithin. On the contrary, I found acetone a very convenient means of producing membrane elevation. Eggs placed in a solution of 3 or 4 c.c. acetone + 25 c.c. of sea-water push out membranes, and cytolysis follows. A very rapid action results if the eggs are placed in more concentrated solutions of acetone.[5] This can be observed by dropping some eggs into a drop of acetone under the microscope.

Chloretone.[6]—This substance is very effective in producing a lowering of surface tension. I found that a 0.1 per cent. solution lowered the surface tension of water by almost one fourth of its value. As was expected, therefore, membrane elevation was produced when a few crystals of chloretone were added to sea-water containing eggs.

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[1] J. Traube, *Liebig's Annalen*, CCLXV., 27.

[2] In 1905, Loeb (Univ. Cal. Publ. Physiology, II., 113) obtained "practically negative" results on adding these acids to sea-water. In 1909 (*Biochem. Zeitsch.*, XV., 254), he made up his solution in $M/2$ NaCl, which in itself has been shown to cause membrane elevation, and in this way he met with occasional success.

[3] *Loc. cit.*

[4] Loeb, "Chemische Entwicklungserregung," p. 105.

[5] Solutions of acetone in sea-water are not as concentrated as they seem, for the acetone tends to be salted out of solution. (See Bernthsen, "Organische Chemie," p. 170.) The surface tension of acetone at 16.8° is 23.35 dynes per centimeter.

[6] Chloretone is a trade name for tri-chlor tertiary butyl alcohol.

Urethane.—According to an approximate determination, a 0.5 M solution of urethane has a surface tension of about 61.3 dynes per centimeter, a value considerably below that of pure water. Hence all solutions of urethane produce membrane elevation (and cytolysis) whether they be isotonic, hypotonic, or hypertonic. Solutions of urethane in sea-water likewise give results, although this substance is not as effective as chloretone.

Chloral Hydrate acts in similar fashion to chloretone and urethane.

Esters.—All esters possess the property of lowering surface tension.[1] The following esters were used and proved effective in producing membrane elevation. Methyl acetate, ethyl acetate, ethyl butyrate, methyl salicylate.

The substances which lower surface tension are very numerous Not all, however, produce membrane elevation. In general, there are three classes of exceptions.

1. *Colloids.*—Many proteins lower surface tension but do not cause membrane elevation.

2. *Protein Coagulants.*—If the solution is strongly coagulative, no sign of membrane elevation will appear. However, if the coagulative action is not very strong, or if the surface tension is quite low, membranes are often pushed out. Under these conditions, a number of little elevations, the so-called "bubbles" or "blisters" are often seen to surround the egg. Loeb and his pupils consider these "blisters" as a preliminary stage in membrane elevation, but they can be observed only when a protein coagulant is present. By adding protein coagulants and at the same time lowering the surface tension, "blister" formation can always be induced. For example, if the eggs are subjected to a solution of acetamide, which contains free acetic acid, they immediately become surrounded with blisters. Similar results are gained with aqueous solutions of picric acid. If the surface tension is low enough, membranes sometimes are formed.

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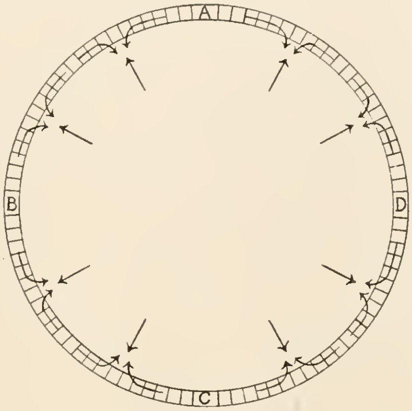
[1] Cf. J. Traube, *Ber. d. deutsch. chem. Gesellsch.*, XVII., 2294 (1884).

3. *Sugar and Glycerin.*—A solution of cane sugar possesses a surface tension very slightly above that of pure water,[1] a glycerin solution has a surface tension below that of sea-water. Thus, both of these solutions have a lower surface tension than sea-water, but if eggs are placed in them, no membrane elevation occurs. The reason for this exception will be considered shortly.

I have shown that membrane elevation depends upon a lowering of surface tension, and I shall now consider the mechanism of the process. As has already been pointed out, the unfertilized egg is surrounded by a membrane, which is probably a gel or semi-gel. In the appended diagram, *ABCD* represents this gel, whose parts pull on each other by reason of their surface tension. As a result of this pull, the underlying egg contents, and more especially the peripheral portions of the egg, are under pressure. This pressure inward is compensated by a "Quellungsdruck" due to a tendency of the proteins to swell. Upon a diminution of surface tension, the pressure inward is relaxed and the protein or proteins directly beneath the membrane immediately swell, thus pushing out the membrane. If the surface tension remains lowered, the entire egg swells, and cytolysis results.



It was found that not all lowerings of surface tension produced membrane elevation, there being three types of exceptions. The reason for these exceptions can now be explained. Although many colloids lower surface tension, they are unable to diffuse into the membrane, and thus lower its surface tension. Moreover, because of their inability to penetrate the membrane, they exert pressure against it, osmotic or otherwise. Secondly, coagulative agents prevent membrane elevation, since they make it impossible for the protein which causes the phenomenon to swell. Probably the main reason that glycerin and sugar solutions do not produce membrane elevation is their action in increasing the viscosity of the gels.[1] When the viscosity of the membrane is augmented, it loses its fluidity and can no longer be pushed out. This hardening effect can be observed directly by placing fertilized eggs with membranes in a sugar solution and examining the result under the microscope.

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[1] Forch, *loc. cit.*

When acids are used to produce membrane elevation, a return to sea-water is usually necessary. No doubt, the acid by virtue of its coagulative power inhibits membrane elevation. On a return to sea-water the coagulative effect is lost by dilution, but enough acid has probably been adsorbed by the membrane to lower its surface tension sufficiently for membrane elevation.

For the sake of simplicity, one of the factors which must have an influence on membrane elevation has been omitted from the discussion. It has been shown[2] that in the presence of chlorides, bromides or nitrates, gelatine shows a greater tendency to swell than in pure water, but that sulphates, sugar, and glycerin have a retarding effect. If membrane elevation is the result of the swelling of a colloid, and if this colloid behaves as gelatine, the presence of chlorides, bromides, and nitrates would accelerate the process, whereas sugar and glycerin would retard or prevent it. The effect of these substances is probably of secondary importance.

In the course of the argument, evidence has been adduced which may also serve to explain other biological problems. Although the toxicity of distilled water has often been noted, no one has ever offered a satisfactory explanation. Bullot[3] found that the purest water obtainable (redistilled in platinum and quartz) was toxic to the fresh water *Gammarus*. It is believed that this toxicity is due to the lower surface tension possessed by pure water (see p. 350). The experiments of R. Lillie have often been adduced as evidence in favor of the "theory of antagonism." Lillie's results find a most suitable explanation when the surface tension of the solutions is considered (see p. 351). A recent paper by Lillie[1] seeks to show an antagonism between salts and anæsthetics in their action on starfish and sea-urchin eggs. This would seem to contradict the results gained in this paper. All that Lillie shows, however, is that various anæsthetics, such as ether, chloroform, etc., exert a slight protective influence on eggs partially cytolyzed with isotonic NaCl solution. This protective action is probably due to the effects of the anæsthetics upon the bacteria which are always found to infest cytolyzing eggs. When these are killed the egg is able to live longer. Gorham and Tower[2] showed that sea-urchin eggs could be kept in healthy condition in sterile sea-water for 11 days or longer, whereas they soon disintegrate if exposed to bacteria.

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[1] Leick, *Drude's Annalen*, XIV., 139 (1904).

[2] See Freundlich, "Kapillarchemie," p. 512.

[3] G. Bullot, Univ. Cal. Pub. Physiol., I., 199 (1904).

SUMMARY.

1. All known methods of producing an elevation (formation) of the vitelline membrane in the egg of the sea-urchin result in a lowering of surface tension.

2. The following substances, all of which lower surface tension, were also found effective in producing membrane elevation: acetone, chloretone, urethane, chloral hydrate, methyl acetate, ethyl butyrate, methyl salicylate, acetamide, picric acid.

3. A simple physical explanation of the process is given, which is based on Fol's original interpretation.

I desire to express my sincere thanks to Prof. B. F. Kingsbury of Cornell University for helpful advice in the preparation of this paper. Thanks are also due to Prof. W. D. Bancroft, who has been consulted on several questions of a chemical nature.

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[1] R. S. Lillie, *Amer. Journ. Physiol.*, XXX., 1 (1912).

[2] F. P. Gorham and R. W. Tower, *Amer. Journ. Physiol.*, VIII., 175 (1902).

EXPERIMENTS ON COÖRDINATION AND RIGHTING IN THE STARFISH.

LEON J. COLE.

In another paper (Cole, '13) I have presented and discussed the results of a series of experiments on the direction of locomotion of the starfish, *Asterias forbesi*, with respect to morphological axes, in the absence of directive stimuli. The experiments described in the present note were incidental to the more complete series just mentioned. They were performed tentatively to test the feasibility of certain lines of investigation which suggested themselves in the course of the other work, but which I have been unable to prosecute further. While they are not extensive enough to serve as a basis for far-reaching conclusions, they nevertheless supplement to some extent the observations of others, and, it is believed, suggest certain lines of research which might be followed up with profit. All of the experiments here described were made in November, 1909, in the animal behavior laboratory of the department of zoölogy, Sheffield Scientific School of Yale University.

In the locomotion of the starfish it was found that the "unified impulse" was an important factor, and Jennings ('07) had previously determined the same to be true in the righting reactions of the West Coast starfish, *Asterias forreri*, which he studied. The establishing of a "unified impulse" toward performing a definite action, such as turning on certain rays, or crawling in a certain direction, implies, of course, coördination. Loeb ('00) in his book on the "Physiology of the Brain" lays stress on coördination in the starfish, though apparently merely as an inhibiting influence, which prevents the rays not helping in a given reaction from working against its consummation, rather than one which causes them to join actively in bringing it about, that is, by taking part in a "unified impulse" toward that end. In discussing, for example, an experiment which will be mentioned later, he says (p. 63): "The experiments seem to indicate that in a normal starfish the stimulus produced by the pulling of two or three arms in the same direction has an inhibitory effect on the other arms." Again (p. 65), he explains the movement of a starfish in a direction away from a ray which has been stimulated by saying that "the feet of this arm are drawn in and the arm becomes inactive," and "therefore, according to the parallelogram of forces, a movement away from the point of stimulation will take place." While stimulation of an arm may cause temporary inactivity of the feet of that arm and this might aid in initiating movement in the opposite direction (Moore, '10), the careful observations of Jennings ('07, p. 97) seem, however, to show that this explanation is only partial. He remarks on this point: "It is possible that in some cases when one ray is stimulated locomotion takes place entirely with the other rays, but such cases are very rare; though I have watched carefully for this, I have never seen one. As a rule the walking away from the stimulated region is due, like the usual locomotion of the starfish, to the coöperation and coordination of the tube feet of all the rays . . . . The active tube feet of all the rays are pushed forward in the direction in which the starfish is going; their suckers attach themselves, and by the contraction of the tube feet . . . the starfish is carried forward, the action of all the tube feet aiding in this." My own observations agree in this respect with those of Jennings. It is therefore evident that in locomotion as in righting an impulse is established, by which the different parts act in more or less complete harmony toward the accomplishment of a certain result. It is generally assumed, and is undoubtedly true, that this unity of action is made possible by the mediation of the nervous system.

Section of Radial Nerves.

The question naturally arises, however, whether it is not possible that an impulse toward the accomplishment of a definite end might be established without nervous connection between the different parts of the organism, just as an earthworm continues its coördinated locomotor contractions after section of the ventral nerve cord, or indeed if the worm itself be cut entirely through and the halves united by thread (Friedländer, 1888).

Here the muscular pull of the forward part acts as the stimulus for contraction in the posterior piece. Loeb's experiment in this connection was negative. He cut the nerve ring of a starfish at two points, nearly opposite to each other, thus severing the nervous connection of certain arms with the others. He found that whereas "the normal starfish requires but a few minutes to turn over, . . . the specimen [operated upon as described] remained on its back the whole afternoon, although the arms were struggling constantly to right it" (Loeb, '00, p. 63). Romanes ('85, p. 296) had, however, previously obtained different results in his experiments on the common British starfish. He states that when animals with the radial nerves severed at the bases of the arms are inverted, "the power of effecting the righting manœuvre is seen to be gravely impaired, although eventually success is always achieved." My experiments on *Asterias forbesi* agree in this respect with those of Romanes.

Experiment 1. (Specimen No. 10 of earlier paper.)—In three preliminary trials this specimen required 6 minutes, 5 minutes and 6 minutes respectively for righting, and turned on arms *cd*,[1] *bc* and *cd* in the successive trials. An incision was now made at the base of each arm, thus severing the radial nerves (and of course the radial water canals as well) close to their origin from the circumoral ring. The specimen was again placed on its back in the water, and the various arms at once began to make individual and apparently random movements toward righting.[2] While most of the arms went through various activities, bending and twisting, attaching and pulling, only to let go again, arm *c* persisted only in bending up orally, and did not twist and attach. This resulted finally, at the end of 20 minutes, in its becoming bent over far enough to obtain a hold between *a* and *e*, as shown in Fig. 1*A*. Arms *b* and *d* were also thrown well over at the same time, but were not attached. Soon these latter straightened out again and the attachment of *c* was loosened. Twenty minutes later *c* and *d* bent over together and succeeded in obtaining a hold between *b* and *a* (Fig. 1*B*). By their concerted effort they succeeded in pulling the body over, *e* crossed over *a* (Fig. 1*C*), and at the end of 44 minutes from the time it was placed on its back the starfish had completely righted itself, turning, as seen, on arms *a* and *b*. It now remained quiet for two or three minutes, and then began crawling with *b* in advance, the movement being, however, very slow, as if there were not complete coördination of all the arms.

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[1] Following Jennings ('07) the arms are designated *a*, *b*, *c*, *d* and *e*, beginning at the ray to the right of the madreporite and going around clockwise (cf. Cole, '13, p. 2). Dr. R. T. Jackson has emphasized (*in litt.*) the desirabilty of students of animal behavior, as well as specialists, using for the starfish the nomenclature introduced by Lovên for the ambulacral and inter-ambulcral areas of Echini. With this view I am in accord and should have adopted the method had it not been that my earlier paper necessitated so much direct comparison with that of Jennings, and a different nomenclature would have made such comparison difficult.

[2] If there is any shock from the operation, it is so slight as to be hardly noticeable.

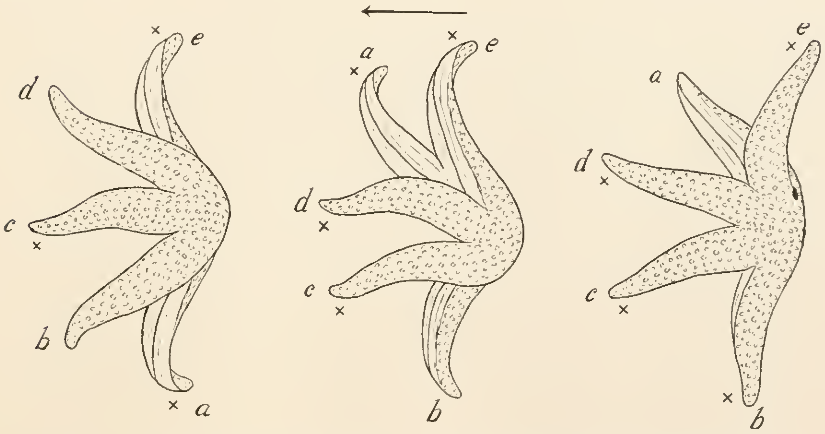

FIG. 1. Diagrams of righting movements of a starfish having the radial nerves severed at the bases of all the arms. Arms attached to substratum are indicated by ×. Arrow shows direction of turning.

Experiment 2. (Specimen No. 14.)—In eleven preliminary trials this animal righted itself in from one to five minutes, the average being 2.6 minutes. In ten of the trials it turned on arms $a + e$. An incision was now made on the oral side at the base of each arm, severing not only the radial nerve and water canal, but also the muscles and other internal organs, and leaving only the aboral arch of the external skeleton intact. It was now placed on its back in the water. It is unnecessary to give in detail the movements of the arms; it is sufficient to say that they at once began active but entirely uncoördinated movements.

Nevertheless, at the end of 56 minutes the animal had succeeded in righting itself, though arm *b* was still crossed over arm *a*. At the end of one hour, however, from the time it was placed on its back, it had straightened out completely. The turning was accomplished with arms *e* and *a*, although *c* swung over between them and really seemed most instrumental in pulling the animal over.

In both of these experiments the lack of coördination was very apparent, as mentioned by both Romanes and Loeb. Neither could it be determined that in either experiment a definite impulse toward turning in a given direction was established, as is normally the case. In fact, especially in the second experiment, it was noticed several times that as two or more arms happened at the moment to be working in concert, the righting could have been effected easily if another arm had but ceased its efforts in an opposite direction, to say nothing of helping in the same direction. The final turning appeared to be nothing but a chance occurrence when enough arms happened to be pulling in one direction to turn the specimen in spite of its other arms. Thus, although there resulted complete lack of coördination, as Loeb states is the case when the nerve connections are severed, the end result was nevertheless finally accomplished, apparently by accident. The pull of one arm on another does not seem, therefore, to be a sufficient stimulus to induce a definite coordination of the tube feet in the second arm.

The second specimen did not crawl after righting itself. It would be interesting to test whether by exerting a steady pull in one direction on such an "uncoördinated" specimen, a more or less unified impulse to crawl in that direction might not be induced, comparable to the crawling of the severed earthworm.[1] This experiment was not tried, but the lack of coördination shown in the righting would make it seem that a negative result might in all probability be expected.

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[1] These experiments might be made even more comparable by severing the rays of the starfish completely from the disc and then sewing them in position again with thread.

Righting of Specimens with One or More Arms Removed.

The removal of one or more arms gives opportunity to test and observe the correlation in the remaining arms more closely. This study was not carried far, and a single example will suffice.

Experiment 3.—A starfish was tested a number of times and it was found that it turned regularly on arms *e* + *a*. Arms *b* and *d* were now severed completely at the base. If the animal now continued to turn on *e* and *a*, the only other arm concerned would be *c*, and it was expected that this would each time release as soon as coördination was established, allowing *e* and *a* to pull it over. It was proposed then to sever the nerve of *c* in order to destroy its coördination with the other arms, and to see if they combined would still be able to pull it over, or whether coördination might be reëstablished through the mechanical pull, without nervous connection. In the first two trials after the operation the specimen turned on *e* and *a* as expected (Fig. 2), but in the third trial *c* failed to coördinate properly and finally it with *a* pulled over *e*, which was doubled under until these arms had crawled far enough to enable it to straighten out.

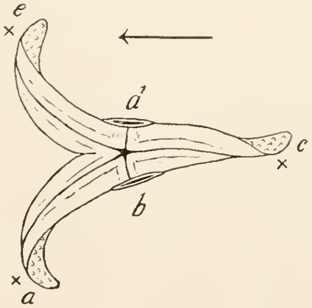

Fig. 2. Diagram of righting of a starfish with arms *b* and *d* removed. Nerves of other arms intact.

This experiment is not mentioned so much for the result obtained as to illustrate a possible method of studying coördination, impulses, and the relative use of the different arms in the starfish. It is important that the behavior of each specimen should be studied carefully in the normal condition before the operation is made.

Behavior of Completely Severed Arms.

Romanes ('85, p. 294) found that "single rays detached from the organism crawl as fast and in as determinate a direction as do the entire animals," and that "when inverted, separated rays right themselves as quickly as do the unmutilated organisms."

The question naturally arises, however, in the light of Jennings' observations that there is commonly a preference for the use of certain rays in righting, whether these same rays will be the first to turn if all are detached and inverted, and whether they will twist in the same direction as when helping to right the entire starfish. Will subsequent locomotion also be in the direction with respect to each of the arms that it would have been if the arms were attached?

The detached arms have two principal methods of righting themselves when inverted. The first is to curl up orally until they topple over one way or the other, when the tube feet attach and quickly right the arm. The other method is for the tip of the arm to twist until the tube feet can attach. They then start to crawl and the remainder of the arm is soon pulled over much after the manner in which a flatworm rights itself (Pearl, '03, p. 673). A sufficient series of experiments was not performed to answer the questions propounded above, the results so far as obtained being somewhat contradictory. More extended experiments, however, would probably lead to more definite results.

Conclusions.

As stated, the experiments mentioned above were rather incidental to other experiments on the starfish already reported (Cole, '13) and preliminary to others which it was hoped to continue. They are reported now not so much for the value of the results obtained as in the hope that they may stimulate further work along the same line. They do, however, seem to demonstrate, in so far as they go, the failure of the establishment of coördination or "unified impulses" in the starfish by direct pull of one part upon another when the nervous connection between these parts has been severed.

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A NOTE ON THE SPERMATOGENESIS OF TENEBRIO MOLITOR.

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This study was suggested to me by Dr. Nettie M. Stevens, to whom I am deeply indebted for direction and help during the earlier part of the work, carried on while fellow in biology at Bryn Mawr College in 1912.[1]

The form studied is the common meal-worm, *Tenebrio molitor*. The worms were kept in the laboratory in a glass jar, and the testes dissected out as needed in the progress of the work. Five methods of fixation were used: Flemming's strong solution, Hermann's platino-acetic-osmic preparation, Gilson's mercuro-nitrate, and Bouin's fluid. After Flemming or Hermann fixation the testes were stained with iron-hæmatoxylin, and with safranin followed by gentian-violet or lichtgrün; after Bouin, with iron-hæmatoxylin and with thionin; after mercuro-nitrate, with Auerbach's fuchsin-green, with thionin, and with iron-hæmatoxylin.

The safranin and lichtgrün staining after either Flemming or Hermann fixation gave the most satisfactory results. By this method the structures of the nucleus were more clearly differentiated than by any of the other staining methods. The iron-hæmatoxylin and the thionin after any of the fixing agents were next in favor, but they were both useless for some of the most important stages, as they stained linin and chromatin alike. Two of the iron-hæmatoxylin slides, however, were destained sufficiently to give a faint differentiation in that respect. Auerbach's stain was a complete failure with my material; and although Miss Stevens and I both tried the Benda method repeatedly, in no instance did we obtain a typical stain. In two slides of Miss Stevens's the differentiation of the mitochondria was fair, though by no means typical, the mitochondria being stained violet, the chromosomes a deep purple.

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[1] Owing to Miss Stevens's sudden illness and death the work has had to be completed without her supervision. It is only through the great kindness of Dr. T. H. Morgan and Dr. E. B. Wilson, of Columbia University, that I have been enabled to prepare it for publication. My many thanks are due them both for their help and direction.

In 1905 Miss Stevens investigated the spermatogenesis of *Tenebrio molitor* (05*a*), paying special attention to the *X* chromosome. Her only reference to the conjugation stage is one short sentence: "A brief 'synapsis' or condensation stage occurs at the close of the last spermatogonial mitosis." She gives one figure of this stage and one of a slightly later stage, both drawn from iron-hæmatoxylin material. The drawing of the condensation stage shows a dense mass of chromatin at one side of the cell. The other figure shows what I have called the "large-loop" stage.

These figures and the accompanying descriptions indicate that Miss Stevens at that time supposed the conjugation to be of the type described by her for three of the Coleoptera and two of the Lepidoptera ('06*a*) and for *Diabrotica vittata*, another of the Coleoptera ('08). The type as described for these forms was telosynaptic. The very short loops that appear in the synizesis stage, "a prolongation of the last spermatogonial telophase," later straighten out and unite end to end to form loops which at first very often have knobs or slight irregularities at the point where the two chromosomes have come together. These loops then pass directly over into the diffuse spireme.

The appearances in my material indicate a considerable divergence from this type.

In the spermatogonia of the meal-worm there are nineteen large chromosomes and one small chromosome, making twenty in all, as shown in metaphase in Fig. 1. Figs. 2, *a* and *b*, show two views of a spermatogonial telophase from a cyst surrounded on three sides by spermatocytes. I have inclined to believe this stage the last telophase preceding the maturation divisions.

The next succeeding stage that I was able to find is shown in Fig. 3; this cell came from a cyst in which every cell except the one figured showed a dense closely massed clump of chromatin at one side of the cell. This appearance is so usual and so well known I did not think it necessary to show a figure of it. It is very similar to the stages called variously "synizesis" and "condensation," and as in Miss Stevens's material, seems to follow directly on the last spermatogonial telophase. There may be between these two stages such intermediate conditions as Dr. Wilson found in *Lygæus* and *Oncopeltus* ('12). The presence of such stages would probably change the interpretation I have put upon the stages described. I was unable, however, to find any such intermediate conditions, and so have been forced to hold to the present interpretation.

These contraction stages were very common in my material, but the section figured, which I have interpreted as a cross section of such a clump, is a rather rare occurrence. This is to be expected, since sections through many different planes would give a side view of a mass crowded at one side of a cell, while a section through one plane only would give such a polar view as is shown in Fig. 3.

I have interpreted this contraction phase as the stage at which conjugation takes place, thus placing synapsis much earlier in the process of spermatogenesis than Miss Stevens had done in her earlier work. According to this interpretation, in Fig. 3, chromosomes *a* and *b*, *c* and *d*, *e* and *f*, *g* and *h*, *x* and *y*, are cross sections of pairs of chromosomes not yet united; chromosomes *j* and *k*, *l* and *m*, *o* and *p*, are side views of such pairs; and *q* and *r* are the tops of the thickened loops formed by two joined chromosomes.

Miss Stevens followed the work up to this point, and it was under her direction that this stage came to be interpreted as the conjugation stage. It was her opinion that the type here was telosynaptic.

As the side view of this stage shows only a very dense, irregular chromatin mass, and the cross sections of this stage are so rare, the actual meeting of the two chromosomes would be very difficult to see; I did not succeed in finding it. But the fact that at the end of this stage never more than ten chromosomes can be found in a cell, while just before it (that is, in the last spermatogonial telophase) twenty were present, is in itself proof that conjugation takes place at this time. And the appearance of the short loops (Figs. 4, 5, 6, 7, 8) as the condensation stage opens up, is an indication of the manner in which synapsis has occurred.

The next stage in the observed process is the transformation of these short thick loops into long loops, partly linin, partly chromatin. This, I believe, has not been hitherto described for any form and is the phenomenon so effectively brought out by the safranin-licht-grün process of staining, the linin staining a clear green, the chromatin a deep rich red. By no other staining method that I used was this differentiation clearly brought out. In almost all preparations these loops appear to be homogeneous, as Miss Stevens has drawn them in her paper ('05*a*). Figs. 5, 6, 7, and 8 show several stages in this process of loop-formation. How this transformation is brought about I did not determine. At this stage there is usually some amount of green-staining material mixed in with the chromatin mass. Whether the linin of the loops comes from the chromatin or from this diffuse green-staining material I did not determine.

There are always ten or fewer chromosomes in sections of cells at this stage, which is the period of general growth. The chromatin at the tops of the loops thickens and becomes dumbbell-shaped, and both the cell and the nucleus nearly double in size (compare Figs. 8 and 13).

Toward the end of this period of general growth, the loops begin to straighten out and then to become attached to each other by their linin ends, thus forming the spireme (Figs. 9, 10 and 12). The spireme never becomes diffuse, but retains this appearance of dense chromatin masses, like dumbbells, strung upon a linin thread. This thread gradually becomes fainter and more slender, until at the stage just preceding the first spermatocyte division, it is seen as an almost indistinguishable double line connecting the chromosomes (Fig. 13). Rarely during these later stages can a chromosome be found in a tetrad form (Fig. 12).

Fig. 14 shows these ten bivalent chromosomes in the prophase of a first maturation division, which is the reductional division.

CONCLUSIONS.

1. No stages were found intervening between the last spermatogonial telophase and the contraction stage.

2. When the chromosomes have fully emerged from the contraction stage, they are in the form of loops, and are in the reduced number.

3. The loops formed by these bivalent chromosomes are not homogeneous, but are partly linin.

4. No diffuse "resting stage" was found, the chromatin being in the form of compact, definite bodies throughout the whole observed process.

5. No diffuse spireme was found.

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EXPLANATION OF PLATE I.

FIG. 1. Flemming; safranin and licht-grün. Spermatogonial metaphase, 20 chromosomes.

FIGS. 2*a* and *b*. Ibid. Two views of a spermatogonial telophase.

FIG. 3. Flemming; safranin and licht-grün. Cross section of contraction stage; *a* and *b*, *c* and *d*, *e* and *f*, *g* and *h*, *x* and *y*, pairs of chromosomes in cross section, not yet united; *j* and *k*, *l* and *m*, *o* and *p*, pairs of chromosomes in side view, ready to unite; *q* and *r*, tops of short loops formed by the thickening, shortening and thickening of two united chromosomes.

FIG. 4. Flemming, safranin and licht-grün. A little later stage in the contraction phase, showing loops.

FIGS. 5, 6, 7, 8. Flemming, safranin and licht-grün. Formation of large loops.

FIG. 9. Flemming, iron-hæmatoxylin. Large loops beginning to straighten out.

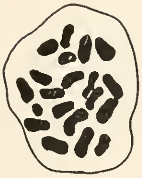
FIG. 10. Hermann, safranin and licht-grün. Formation of spireme.

FIG. 11. Hermann, safranin and licht-grün. Showing the ten chromosomes of this stage.

FIG. 12. Hermann, safranin and licht-grün. Spireme, showing tetrad.

FIG. 13. Flemming, safranin and licht-grün. Late spireme.

FIG. 14. Hermann, safranin and licht-grün. Prophase of first spermatocyte division, showing ten bivalent chromosomes, including the unequal pair.



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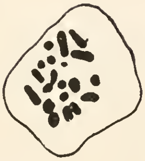

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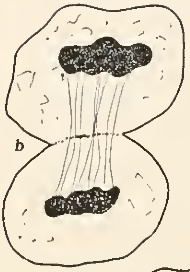

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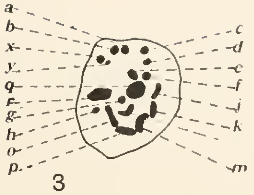
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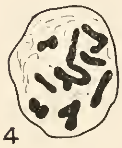

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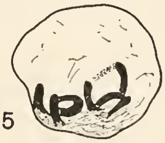

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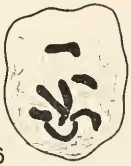

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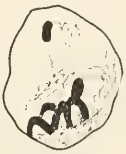

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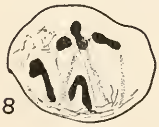

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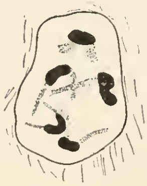

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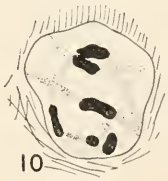

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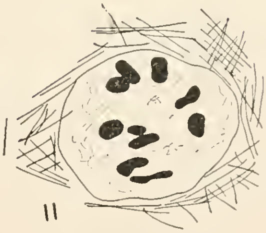

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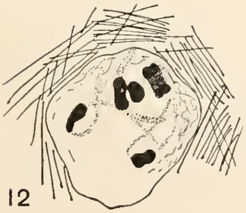

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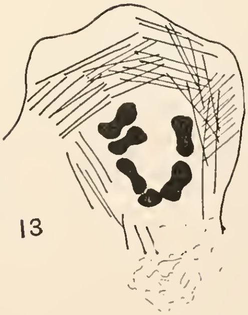

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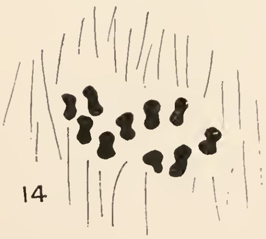

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R. J. STOCKING.

Vol. XXIV. *May, 1913.* *No. 6.*

BIOLOGICAL BULLETIN

SOME ANOMALIES IN THE GESTATION OF THE ALBINO RAT (MUS NORVEGICUS ALBINUS).

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In the course of a series of inbreeding experiments with the albino rat which have been in progress for several years, records have been made of the births of over 700 litters containing some 5,000 individuals. These records show a number of striking irregularities in gestation which are deemed worthy of note, since at the present time the albino rat is extensively used as a laboratory mammal in many of the large institutions of the country.

As the data given in this paper form part of the permanent records on file at The Wistar Institute, it has seemed advisable to refer to individual rats by the numbers that will identify them on the record cards, instead of employing some conventional lettering. The scheme for designating the rats, which is outlined below, has been found very satisfactory for keeping track of a large number of individuals, since it tells at once the pedigree of any particular animal. In the scheme of marking used the serial letter, *A* or *B*, indicates that the individual was a descendant of one or of the other of the two females, *A* and *B*, with which the experiments were started in the spring of 1909. The serial letter is preceded in all cases by a number which shows the generation to which the rat belonged. An index number, following the serial letter, indicates in which of its mother's litters the animal was born: if no index number is present the rat was a member of its mother's first litter. The subscript following the serial letter is the number that serves to distinguish each particular rat from the other individuals belonging to the same generation and litter group. An illustration of this method of designating the rats will, perhaps, make the scheme somewhat clearer. $6B_{57}^{2}$ for example, denotes a female belonging to the sixth generation of rats that descended from female *B*. She was a member of the second litter borne by her mother, and her individual number in the series of rats belonging to the second litters of the sixth generation was 57.

It is part of the daily routine work in the rat colony to examine the cages containing breeding animals and to record the births of litters. The birth of a litter is ascribed to the day on which the litter is discovered, unless it is evident from the appearance of the young rats that their birth had been overlooked at a previous visit. In the latter case an approximate date is assigned for the birth of the litter, and on the record cards this date is followed by an interrogation mark. As the rat colony is usually visited in the morning it is very probable that, in the majority of cases, a litter is discovered shortly after its birth, since observations made at various times indicate that in the rat parturition occurs most frequently in the morning, although it may take place at any time during the day. All of the dates of the births of litters given in the present paper are correct within a few hours, since no cases have been included in which a litter was obviously more than a day old when discovered.

As a rule the male is removed from the breeding cage just before, or immediately after, the birth of a litter in order to guard against the possibility of his destroying the young rats. This is seemingly a needless precaution, since infanticide is comparatively rare in albino rats, although it is very common among brown rats kept in captivity according to the observations of Miller ('11). When young albino rats are destroyed it is done, as a general thing, by the female, either because her nest is disturbed during parturition or because she is not in a physical condition to suckle her offspring and is annoyed by their attempts to obtain food. In only one case, as yet, have I found a male eating the young, and in this instance the female was equally as guilty as her mate.

The anomalies in gestation that are noted in the present paper are of three kinds: (1) Prolongation of the period of gestation; (2) cases of superfecundation; (3) cases of superfœtation.

1. Prolongation of the Period of Gestation.

According to a series of unpublished records kindly furnished me by Dr. J. M. Stotsenburg, of The Wistar Institute, the time between the copulation of a non-lactating albino rat and the birth of her litter varies from 21 days and 15 hours to 22 days and 16 hours. The normal period of gestation for the albino rat, therefore, can roughly be estimated at from 21 to 23 days. In the brown rat kept in captivity the period of gestation seems to be somewhat longer than in the albino rat, as Miller found that it varies from 23½ days to 25½ days in different cases.

Daniel's ('10) investigations show that in the mouse the period of gestation, which is normally 20 days, is considerably prolonged if the female is suckling young. From data obtained in ten cases he formulates the following law: "The period of gestation, in lactating mothers, varies directly with the number of young suckled." In the course of my experiments records have been made of the births of 31 litters borne by lactating albino rats. These records, as shown in the three following tables, indicate that the length of the period of gestation is affected by the number of young suckled and by other factors as well.

Table I. shows the length of the period of gestation when lactating females were suckling five or less young and carrying five or less young.

Table I.

Number of Female.	Date of Birth, First Litter.	No. Young Suckled.	Date of Birth, Second Litter.	No. Young Born.	Gestation Period, Days.
$5A_{2}$	July 14	3	Aug. 8	5	21
$2A^{2}_{1}$	Aug. 26	4	Sept. 9	5	21
$5A_{27}$	Dec. 28	5	Jan. 18	4	21
$7A_{76}$	June 21	5	July 13	5	22
$4A_{15}$	May 23	4	June 6	3	23
$8A_{8}$	Aug. 14	4	Sept. 9	5	23
$5A_{56}$	Nov. 15	5	Dec. 8	3	23
$4B_{24}$	June 14	5	July 7	5	23

In each of the eight cases cited above the period of gestation can be considered as normal since it did not exceed 23 days. It appears, therefore, that the period of gestation in lactating albino rats is not extended if the number of young suckled and the number of young in the second litter does not exceed five in either case.

In Table II. are given the data for cases in which the number of young suckled was five or less while the number of young in the second litter exceeded five.

TABLE II.

Number of Female.	Date of Birth, First Litter.	No. Young Suckled.	Date of Birth, Second Litter.	No. Young Born.	Gestation Period, Days.
$2B^2_1$	June 8	3	June 29	7	21
$1B_1$	Jan. 15	3	Feb. 5	8	21
$6A_{83}$	April 24	5	May 17	8	23
$4B_{13}$	June 2	4	June 26	6	24
$4A_{29}$	Dec. 20	4	Jan. 14	7	25
$5A_{26}$	Nov. 20	4	Dec. 14	9	25
$8B_{32}$	Aug. 20	3	Sept. 15	13	26
$5B^2_4$	Aug. 30	3	Sept. 26	8	27
$4B_{33}$	Aug. 3	4	Aug. 30	8	27
$3A_3$	Jan. 15	4	Feb. 12	7	28
$5B^2_3$	Sept. 11	5	Oct. 10	12	29

In eight of the eleven cases shown in Table II. the period of gestation was prolonged from one to six days. In the three remaining cases the gestation period was normal since it did not exceed 23 days. These results indicate that, as a rule, the period of gestation is prolonged when a lactating female, suckling a small number of young, is carrying a litter containing six or more embryos.

Table III. shows the length of the period of gestation in lactating females suckling more than five young.

TABLE III.

Number of Female.	Date of Birth, First Litter.	No. Young Suckled.	Date of Birth, Second Litter.	No. Young Born.	Gestation Period, Days.
$5A_{13}$	Dec. 8	7	Jan. 2	6	25
$5A_4$	Nov. 25	7	Dec. 20	8	25
$7A_{76}$	Sept. 11	6	Oct. 7	3	26
$5B_1$	July 4	6	July 30	9	26
$4B_{40}$	July 13	6	Aug. 8	10	26
$8A_{75}$	Sept. 16	6	Oct. 13	5	27
$8A_9$	Sept. 13	7	Oct. 11	5	28
$5B_{32}$	Oct. 5	8	Nov. 5	8	30
$8B_{27}$	June 1	10	July 1	8	30
$8A_{51}$	Sept. 13	9	Oct. 14	4	31
$4B_{51}$	July 26	11	Aug. 28	12	33
$6B^2_{5\,7}$	May 24	9	June 27	12	34

Since in the twelve cases recorded in Table III. the period of gestation ranged from a minimum of 25 days to a maximum of

34 days it is evident that the suckling of more than five young prolongs the period of gestation in the albino rat. The length of the gestation period is not, as a rule, directly proportional to the number of young suckled. For, assuming 22 days to be the average length of the gestation period, only three of the twelve cases fulfill the conditions of Daniel's law. In the majority of cases shown in Table III. the length of the period of gestation seems to have been little affected by the number of young in the second litter. For instance, female $7A_{76}$, with six young in her first litter, had a gestation period of 26 days before the birth of a second litter containing three individuals; on the other hand, females $5B_1$ and $4B_{40}$, each suckling six young, also had gestation periods of 26 days when there were nine and ten young respectively in their second litters. In some few cases, however, the gestation period seems to be further prolonged if a lactating female is carrying a very large litter. This is indicated by the fact that the two longest gestation periods yet recorded, 33 and 34 days, were found only when the second litter contained an unusually large number of individuals.

The data given in the above tables show that the period of gestation in lactating albino rats varies from a minimum of 21 days to a maximum of 34 days. The length of the gestation period seems to depend, primarily, on factors that affect the nutritive conditions of the embryos, but it is also influenced, to some extent, by the individual peculiarities of the females. Lactating females may have different periods of gestation when the number of young in each of their litters is the same, as is shown in the case of females $3A_3$ and $4A_{29}$. Each of these females had four young in her first litter and seven young in her second litter, yet the former had a gestation period of 28 days before the birth of her second litter, while in the latter female the gestation period was only 25 days.

A comparison of the data given in Table II. with that shown in Table III. indicates that in different lactating females the period of gestation is not as extended when five or less young are suckled as when this number is exceeded. This rule seems to apply equally well also to the different litters borne by the same female. Female $7A_{76}$, when suckling five young, had a gestation period of 22 days before the birth of a second litter containing five young; when suckling a litter of six the period of gestation was prolonged to 26 days although there were but three young in the second litter. Why the suckling of six instead of five young should invariably prolong the period of gestation is not at all clear. Records of some 800 litters of albino rats show that the average number of young in a litter is six. Seemingly, therefore, the length of the gestation period is prolonged when the number of young suckled equals or exceeds the number that represents the average size of the litter in the species.

Female $6B_{57}^{2}$ had a gestation period of 34 days, the longest period so far observed. This case is rather an interesting one. The nine young born on May 24, 1912, were suckled until June 20, when it was noticed that the female was pregnant. As the young rats were well developed and able to care for themselves they were removed from the cage, although, under ordinary conditions, a litter is allowed to remain with its mother for a month. The female was watched closely, and parturition was found to take place on the morning of June 27. There was thus a period of one week after the removal of the first litter before the second litter was born, although the normal period of gestation had been passed before the first litter was taken away. Had the young rats in the second litter reached the proper stage of maturity for birth when the first litter was removed it is very probable that they would have been born immediately. In this instance, therefore, lactation did not merely delay parturition but it must have retarded the development of the embryos.

The young rats in the second litter were examined shortly after their birth. They were normal in appearance, but they were very small, weighing not more than 4 gm. each. According to Donaldson ('06), the weight of the albino rat at birth varies from 4.2 gm. to 6.5 gm. As the size of the newborn rat depends to a very considerable extent on the number of individuals in the litter, no significance can be attached to the fact that in this very large litter the rats at birth weighed less than 4.2 gm. each. As it was not considered advisable to allow the female to suckle so many young, six of them were killed at once. The others developed normally, and opened their eyes at the usual time, *i. e.*, when 15 days old. The prolonged period of gestation did not make these young rats more precocious in any way as far as could be determined.

In the rat, as in the mouse according to the observations of Sobotta ('95) and of Long and Mark ('11), ovulation followed by copulation must normally take place within a few hours after parturition, since in all of the cases recorded in the above tables the male was removed shortly after the birth of the litter and the female with her young occupied a cage inaccessible to the entrance of other rats. In none of the cases cited in Table I. could lactation have delayed ovulation, since in each instance the period of gestation was of normal length. The manner in which the experiments were conducted seems to preclude the possibility that the prolonged periods of gestation found in many cases were due to a delay in ovulation caused by lactation, since presumably mammalian spermatozoa are not functionally active for more than two or three days after insemination, although, according to Schultze ('66), they can live in the uterus for as much as six days. It seems probable, therefore, that lactation prolongs the period of gestation by influencing the nutritive conditions of the developing embryos. In the lactating albino rat the physiological conditions affecting nutrition seem to be so adjusted that the suckling of a small number of young does not interfere at all with the development of a second litter which also contains a small number of young. When the number of rats suckled equals or exceeds the number that represents the average size of the litter in the species, then lactation appears to lessen the amount of nourishment that the developing embryos receive. It seems to be more essential for the welfare of the species that the suckling young should be well nourished than that the fœtal young should develop at the normal rate. When a pregnant female is suckling a large number of young, the embryos receive less nourishment than is usually given by a non-lactating mother and consequently they develop more slowly. In cases of this kind the period of gestation is prolonged until the embryos have reached the proper stage of maturity for birth. When a female is suckling a small number of young and carrying a large litter, there is apparently no readjustment of the nutritive conditions so that each fœtus will get the nourishment that will enable it to develop in the usual time. The development of ten or of a dozen embryos on nourishment normally used by six means, necessarily, that the embryos will develop more slowly and therefore that the period of gestation will be prolonged.

The causes that determine whether the period of gestation in non-lactating albino rats shall be 21 days or 23 days are as yet unknown. Presumably the size of the litter is a determining factor in these cases as well as in those in which a female is already suckling young. The age and physical condition of the female are also factors that are probably of importance in this regard. A very young female or one in poor condition would not be able to nourish her fœtal young as well as would a more mature female in good condition, consequently the period of gestation would tend to be longer in the former case than in the latter.

The extension of the period of gestation in the albino rat from 21 days to 34 days is not a remarkable phenomenon, since the early observations of Tessier, quoted in detail by Collins ('86), show that the duration of the gestation period in various mammals is subject to considerable variation.

The data furnished by Collins and Daniels give the extremes of gestation periods shown in Table IV.

Table IV.

Mammal.	No. Cases Observed.	Shortest Period of Gestation, Days.	Longest Period of Gestation, Days.	Difference Between the Extremes, Days.
Cows	575	240	321	81
Mares	447	287	419	132
Sheep	912	145	156	11
Swine	25	109	133	24
Rabbits	161	27	35	8
Mice	10	20	30	10

In none of these cases is the duration of the gestation period relatively as long as in some of the cases recorded in Table III., where the gestation period for the albino rat is shown to have been extended more than one half of the minimum normal period. The relatively greater length of the gestation period in the rat may not, however, be of any significance, since it is very probable that the periods of gestation given in Table IV. are not the longest that have been observed in the various mammals mentioned, although I have not been able to find any records that exceed them.

2. Cases of Superfecundation.

As there is much confusion in literature regarding the use of the words superfecundation and superfœtation, it seems advisable to define these terms as they are employed in this paper. Superfecundation is used in cases where two or more ova belonging to the *same* period of ovulation were fertilized by successive matings. Superfœtation, on the other hand, is applied to cases in which ovulation, followed by copulation, occurred during pregnancy and led to the simultaneous development in the uterus of two sets of ova belonging to *different* periods of ovulation.

Although no observations have been recorded regarding the time required for parturition by the rat it is generally assumed that all members of a given litter are born within five or six hours, as is the case in the mouse according to the investigations of Long and Mark and of Daniels ('12). While this rule doubtless holds good in the great majority of cases there are occasional exceptions, as is shown by the following instance.

Female $8B_5$ gave birth to a litter on April 8, 1912. It became necessary to move the mother and young the day after the litter was born. Six young rats were transferred to the new nest and all of them were apparently of about the same size and weight. Three days later the new nest was examined to see if the transference had caused the female to destroy her offspring. This time eight individuals were found in the litter; two of them were decidedly smaller than the rest and they had the appearance of having been born but a very short time. In this instance there was an interval of at least two days between the birth of the first six members of the litter and of the last two.

On examining litters of albino rats that are from six to fourteen days old one occasionally finds that one or more of the young rats are decidedly smaller and more immature than the others. Such small individuals have been considered by many investigators as runts, and their size has been attributed to the fact that, being constitutionally weaker, they were unable to obtain as much nourishment as the other members of the litter and so had not grown as rapidly. The litter of $8B_5$, described above, shows that in some cases the very great difference in the size of the various individuals in a young litter is not due to constitutional weakness on the part of the smaller individuals, but to the fact that the smaller rats were born two or three days after the larger ones.

In the course of my experiments I have found a number of litters, about two weeks old, in which one or more of the individuals were much smaller than the rest. Two instances will serve as examples of such cases. Female $4B_{39}$ gave birth to a litter on October 27, 1911. When the litter was fourteen days old it was examined and found to contain twelve individuals. Ten of the young rats were in a similar condition of development; they weighed from 13.7 gm. to 14.4 gm. each; they were well covered with hair; and their eyes were beginning to open. The other two members of the litter appeared normal in every way; but their eyes were closed; they had comparatively little hair; and they weighed only 9.1 gm. and 9.3 gm. respectively. Judging from their appearance these small individuals were not more than ten days old.

A litter belonging to female $5B_{19}$ was inspected thirteen days after its birth. Nine of the ten young were in approximately the same stage of development, and they weighed from 16.2 gm. to 17.1 gm. each. The tenth individual seemed normal and well nourished, but it weighed only 10.8 gm. and appeared more immature in every way than did the other members of the litter.

The small individuals in both of these litters were earmarked in order to make their identity certain, and they were allowed to grow up. At the end of two months there was no perceptible difference, either in size or in general behavior, between these individuals and the other members of the litter. The relatively small size of these rats when they were two weeks old did not mean, therefore, that they were constitutionally weaker than their fellows but that they were born later.

In litters of albino rats, especially if the litter is very large, it is not uncommon to find individuals that are less vigorous than the other members and that are stunted in their growth.

An instance recently came under my observation in which a male rat in a litter about three months old weighed only 60 gm., while his brothers and sisters all weighed from 130 gm. to 190 gm. each. This small individual was unquestionably a runt, and he was far less vigorous and active than the other rats in the litter. Runts that are found in litters about two weeks old appear *fully as mature* as do the other members of the litter, but they are much less active. In litters like those described above the smaller individuals are always *less mature* than the other rats, although equally as vigorous and well nourished.

In cases of this kind all members of the litter must have developed from ova belonging to the same period of ovulation, since it is very improbable that a second period of ovulation ever follows immediately after the first. There are two possible explanations for these cases. If at some period of ovulation one or more of the ova were unusually slow in maturing they might not be liberated until two or three days after the rupture of the more mature follicles, and so would not be fertilized at the first mating. Such ova might, however, be fertilized at a subsequent mating; for the period of heat in the rat extends over several days and copulations take place frequently during this time, as the investigations of Miller have shown. As the minimum gestation period in the albino rat is 21 days, ova that were fertilized late could not reach the proper stage of maturity to be born with the embryos that developed from the first ova fertilized, and consequently there would be a considerable interval between the births of different members of the litter. According to this explanation such litters are good examples of superfecundation.

There is another possible explanation for these cases, namely, that all of the individuals in the litter were developed from ova that were fertilized at the same mating, but that for some reason, possibly on account of faulty implantation, some of the embryos received less nourishment than the rest and so reached maturity later. Such conditions, it seems to me, would tend to produce runts, and not merely to retard normal development.

On dissecting pregnant females one frequently finds one or more embryos that are much smaller than the rest. While in some instances such small embryos appear normal and are presumably either runts or embryos that have resulted from superfecundation, in the majority of cases they are pathological, probably because of faulty implantation of the ova. If such pathological embryos are ever born with the rest of the embryos they are destroyed at once by the mother, as they are never found among the normal newborn young.

3. Cases of Superfœtation.

It has been maintained by many physicians that ovulation does not occur during pregnancy and therefore that the conception of a second fœtus by a pregnant woman is impossible. Cases seemingly those of superfœtation have been ascribed to the presence of a bifid uterus, or they have been assumed to be the result of a twin pregnancy in which one fœtus was blighted. Undoubtedly many so-called cases of superfœtation can properly be attributed to one or to the other of these causes, but there are a number of well-authenticated cases, such as those cited by Bonnar ('65), which seem explicable only on the assumption that superfœtation can occur in woman under exceptional conditions.

In the lower mammals superfœtation is seemingly of rare occurrence. An examination of the evidence shows that many cases that have been reported as due to superfœtation are unquestionably instances of superfecundation or of blighted ova. A very probable case of superfœtation in sheep was reported by Arrowsmith in 1834, while the observations of Christopher ('86) show that in the cat ovulation can occur during pregnancy and, therefore, that superfœtation in this species is possible, although it is not known to have taken place.

In the course of my investigations on the rat I have found two cases which are seemingly due to superfœtation. Female $4A_{29}$ had a litter of four young born on December 20, 1910. The male was removed when the litter was discovered and the nest was left undisturbed for fourteen days, when the litter was examined for the sex ratio. At this time the four rats born on December 20 were well developed, and they weighed from 15.5 gm. to 16.4 gm. each. In addition to these rats the nest was found to contain seven very small rats that had apparently been born only a short time before as they were bright red in color and weighed less than 5 gm. each. Between the birth of the first four members of the litter and of the last seven there was an interval of about fourteen days.

Female $6A^{2}_{63}$ gave birth to her first litter of three young on February 26, 1912. Thirteen days later the nest was examined and found to contain seven newborn rats that weighed from 4 gm. to 4.5 gm. each. The older members of the litter weighed at this time 10.1 gm., 10.2 gm. and 10.5 gm. respectively. The interval between the birth of the first and of the last members of the litter was in this instance about twelve days. In each of these cases the female with her young occupied an entire cage, so there was no possibility that the newborn rats could have belonged to another female. None of the small rats in these litters were runts, and, with the exception of one that was killed for a museum specimen, all of them were raised to maturity and some were used for breeding purposes.

Female $4A_{29}$ was not examined after her death for any possible malformation of the genital organs. A careful autopsy was made of female $6A^{2}_{63}$, however, and there was no evidence whatever of any abnormality either in the ovaries or in the uterus. This rat had three other litters besides the one described above, and in each of these litters all of the individuals were born during the one period of parturition.

The cases of superfecundation described in the second section of this paper indicate that a single period of ovulation may extend over three or four days, but it is difficult to see how embryos, born at intervals of two weeks, could possibly have developed from ova belonging to the same period of ovulation. To assume that some of the ova liberated at a given period of ovulation were fertilized at once while others remained in the tubes or in the uterus for nearly two weeks before they were fertilized seems unwarranted; for while it is not known how long mature ova can live without fertilization, it is improbable that they can live for more than a few days at most. That part of the ova belonging to a certain period of ovulation should produce embryos that would be born after a normal gestation period of about 21 days while the rest of the ova had their development so delayed that they were born only after a gestation period of about 35 days also seems highly improbable. The most plausible explanation for these cases seems to me to assume that the two ovaries acted independently, ovulation occurring in one ovary some little time before it took place in the other. If copulation followed each ovulation two sets of embryos would develop in the uterus simultaneously, and they would be born at different times, depending on the interval between the two periods of ovulation.

Conditions which retard ovulation from one ovary for a greater or a less period of time and so make superfœtation possible must occur very rarely in the rat, since the two cases described are the only ones that have been found in the course of my experiments.

Summary.

1. The normal period of gestation in non-lactating albino rats ranges from 21 to 23 days.

2. The gestation period in lactating albino rats is of normal length if the female is suckling five or less young and is carrying five or less young.

3. The gestation period may be prolonged from one to six days if an albino female, suckling five or less young, is carrying six or more young.

4. The period of gestation is always prolonged when a female is suckling six or more young. In these cases the number of young in the second litter seems to have less influence on the length of the gestation period than has the number of young suckled; but if both litters are very large the gestation period may be extended to 34 days.

5. In the albino rat ovulation takes place within a few hours after parturition.

6. Lactation does not delay ovulation, but the suckling of a litter that contains six or more young seems to lessen the food supply to the fœtal young and so retards their development.

7. Superfecundation occurs occasionally in the albino rat and causes an interval of two or three days between the birth of different members of the litter.

8. In rare instances ovulation takes place in the albino rat during pregnancy and superfœtation occurs. In two cases of

this kind litters have been produced at intervals of about two weeks.

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A CHROMATOID BODY SIMULATING AN ACCESSORY CHROMOSOME IN PENTATOMA.

EDMUND B. WILSON.

A brief account will here be given of a body that first attracted my attention in the spermatogenesis of *Pentatoma* (*Rhytodolomia*) *senilis* Say, where it offers so deceptive a resemblance to an accessory or unpaired *X*-chromosome that it might readily lead to erroneous conclusions could not its entire history be followed out in every detail. A body that is evidently of the same nature but somewhat smaller was afterwards found in *Pentatoma* (*Chlorochroa*) *juniperina* L., and in *Podisus crocatus* Uhler, where it is still smaller. A similar body is often seen also in *Cœnus delius* Say, but appears here to be of less constant occurrence. Its behavior is essentially the same in all these cases, and I suspect that it will be found in many other insects. It is probably of the same general nature as the bodies that have been described by various observers as "chromatoid Nebenkörper," "chromatoid corpuscles," etc., in the spermatogenesis of vertebrates, insects, and other animals. In *Pentatoma senilis* it is of very large size, invariably present, and almost always single, though one or two similar but smaller granules often appear also in the same cell. Its constancy and conspicuous character in this species exclude the possibility of error in its identification at any period save the earliest.

As seen during the growth-period and the spermatocyte-divisions it is of rounded form, dense and homogeneous consistency, and after double staining with hæmatoxylin or safranin and light green is at every stage colored intensely blue-black or brilliant red, precisely like the chromosomes of the division-period or the chromosome-nucleoli of the growth-period. In the first spermatocyte-division it may lie anywhere in the cell, sometimes almost at the periphery, but is often close beside the chromosomes. In the latter case it usually lies in, on or near the spindle, lags behind the chromosomes during the anaphases, and in later stages is found near one pole, presenting an appearance remarkably like that of an accessory chromosome (Figs. 8–10). For such in fact I at first mistook it, even after the discovery that a similar body is often also seen near one pole in the *second* division (Figs. 22, 23); for I supposed this might be a case like that of *Ascaris megalocephala*, where, according to Edwards ('10) the *X*-chromosome may pass undivided to one pole in either the first or the second division. The resemblance is indeed most deceptive; and these division-figures have often been exhibited to other observers as "a remarkably clear demonstration of an accessory chromosome" without at first arousing the least suspicion of the hoax.

The body in question is nevertheless neither an accessory nor any other kind of chromosome; though this did not become wholly certain until after a study of the entire spermatogenesis. It is in fact of protoplasmic origin, first appearing early in the growth-period outside the nucleus, whence it may be followed uninterruptedly through all the succeeding stages until it is finally cast out of the spermatozoön. Upon dissolution of the nuclear membrane it is left lying near the chromosomes, passes without division into one of the daughter-cells in each of the spermatocyte-divisions, and thus enters but one fourth of the spermatids. In the latter it persists with little or no change until a very late stage, sooner or later wanders far out into the sperm-tail, and is at last cast off altogether. It therefore takes no visible part in the formation of the spermatozoön.

I have as yet been able to study the testes of only a single individual of *P. senilis:* but since the whole history of the chromatoid body is clearly shown in this material the facts will be described because of the emphasis that they place on certain possible sources of error in the study of the numerical and sexual relations of the chromosomes. One of the testes was fixed in Flemming's fluid (the best fixation), one in Bouin's, and both were at first stained in iron hæmatoxylin. One of the Flemming slides was also stained in light green: this slide was afterwards extracted and successfully restained with safranin and light green, giving a preparation of great brilliancy. The results given by these four methods of staining are entirely consistent. In all cases the chromatid body is intensely and purely stained by the "nuclear" dye. In the Flemming material the intranuclear plasmasome, sharply defined and uniformly present, is after hæmatoxylin alone pale yellowish, after both double stains clear green. In the Bouin slides, as is often the case with material thus fixed, the plasmasome stains with hæmatoxylin almost as intensely as the chromosome-nucleolus, and in the earlier part of its history is often indistinguishable from the latter. Unsuccessful attempts were made to restain the Bouin slides with the Biondi-Ehrlich and the Benda (mitochondrial) methods. With the latter, both chromosomes and chromatoid bodies stained alike with the crystal violet after every degree of extraction. With the former method, neither of these bodies could be made to hold the methyl green, both staining alike clear red. I think it probable, however, that with suitable fixation these two kinds of bodies could be differentiated by one or the other method.

I.

Without additional material, including smear-preparations, the interesting history of the chromosomes can not be completely elucidated, owing to certain difficulties in the early and middle prophases. With exception of these stages however the relations of the chromosomes are shown with a diagrammatic clearness that leaves nothing to be desired; and they will be briefly described, both for their own sake and because this is necessary to a complete demonstration of the behavior of the chromatoid body.

The spermatogonial groups (Figs. 1–3) contain six chromosomes, the smallest number thus far recorded in any heteropteran (in *P. juniperina* the number is 14, as in *Euschistus*). Two of these may always be recognized as the largest. Of the remaining four two, as the subsequent history proves, are the X- and Y-chromosomes; but these differ so slightly in size that they can hardly be distinguished with certainty at this time. The first spermatocyte-division may be described as showing in polar view four chromosomes (Figs. 4–6), including one large (B) and one small bivalent (b), and the univalent X- and Y-chromosomes; but the latter lie close together, often in contact, and not seldom are so closely associated that they might well be reckoned as forming a single tetrad. In the second division *X* and *Y* are uniformly coupled to form a bivalent (Figs. 14, 15); hence but three chromosomes appear in polar view (Fig. 13), and each spermatid receives this number.

The relations of these chromosomes in the spermatocyte-prophases and in the first division show some very interesting features. The autosomes (bivalents) are always characterized by their quadripartite or tetrad structure, being deeply constricted transversely, and also longitudinally cleft, sometimes so markedly that in polar view each appears distinctly double (Figs. 5, 6, 19, 20). This cleft, extremely conspicuous in the prophases, is often less marked during the metaphase, but is again conspicuous in the anaphases, when each daughter-chromosome is always distinctly double, as may be seen with diagrammatic clearness in polar views (Figs. 11, 12). The *X*- and *Y*-chromosomes, on the other hand, are always bipartite and only form a quadripartite body when the two are in contact or closely associated. In the middle prophases they lose their compact, nucleolus-like form and become more or less elongated, looser in texture, and they are now conspicuously split lengthwise, but show no sign of transverse division. In later prophases, as all the chromosomes condense, they shorten more or less but still retain, as a rule, the form of longitudinally split rods at the time they enter the metaphase group. They are at this time frequently in contact end to end (Fig. 17), but *may be quite separate*, though always near together. Their later history is shown in Figs. 18, *a–f*, all of which are accurately represented in the same position with reference to the spindle (in correspondence with Fig. 17). Of this series of figures, *a* and *b* are from metaphases, the others from anaphases. This series, every stage of which is shown in numerous cases in the preparations, clearly demonstrates that the original longitudinally split rods progressively shorten, and that the two halves of each are then drawn apart at right angles to the original long axis. Each chromosome thus assumes a dumb-bell shape (*a–c*) and finally divides "transversely"; but it is certain from the earlier stages that this division is only the completion of an original longitudinal split. When *X* and *Y* are closely associated (as in *a* and *b*) they may be described as forming an actual tetrad, the first division of which is evidently equational.

An excellent opportunity is afforded by these stages for critical comparison of the relative sizes of *X* and *Y*. Such a comparison, made in a large number of cells, shows that a slight but evident inequality almost always exists, the fact being placed beyond doubt by its appearance in both daughter-pairs (Figs. 18, *d–f*). The inequality is however not always seen, perhaps because of slight differences of form or foreshortening. In respect to the size-differences between *X* and *Y*, *Pentatoma senilis* seems to approach *Oncopeltus fasciatus*, as described in my eighth "Study," and differs markedly from *P. juniperina*, where the difference is as evident as in *Euschistus*.

Among numerous cases examined I have not found an exception to the rule that *X* and *Y* lie near together, often more or less in contact, and divide in this position. This relation is maintained during the anaphases, so that in the final stages *X* and *Y* form a pair that is hardly to be distinguished from the small bivalent autosome, now always distinctly double (Figs. 11, 12). Each secondary spermatocyte thus receives three pairs of chromosomes, two of which are the longitudinally divided daughter-autosomes and the third the *XY*-pair. These three double elements enter the second division spindle directly, without forming a "resting-nucleus," and each is separated into its two components (Figs. 14–16). This of course involves the disjunction of *X* and *Y*. In this division the inequality between these chromosomes is almost always in evidence, the appearance of the *XY*-pair being somewhat similar to that of *Nezara hilaris*, as described in my seventh "Study."

It would be hard to find a more convincing demonstration than is here afforded of the relation between univalents and bivalents in the maturation-divisions (*cf.* Wilson, '12) or of the fact that the bivalent is equivalent to two univalent dyads united together. Were the association of *X* and *Y* only a little closer they would in fact form an actual tetrad essentially like the autosome tetrads. Considering the *XY*-pair as such, it evidently divides longitudinally (equationally) in the first division and in the second undergoes a typical reduction-division (disjunction of *X* and *Y*).

Despite the small number of chromosomes I have not been able to understand completely their history during the early and middle prophases, when their elongate form and close apposition to the nuclear membrane renders their complete analysis in sections very difficult. Smear-preparations alone, I believe, will fully clear up the facts. In the middle prophases (Fig. 31) the chromosomes are characterized by the exaggerated character of the longitudinal split (as compared with other insects I have examined) the two halves being often quite as widely separated as in the copepods (Häcker and his followers). Their number at this time often appears to be *five* (instead of the expected number, four), and in some cases even six, the diploid number. This puzzling fact I can not yet fully explain; but it appears to be due to the very wide separation of the two moieties of one or both bivalents at the point where each is destined to undergo "transverse" division in the first spermatocyte-mitosis. It is at any rate certain that this is characteristic of the large bivalent during the earlier period of its condensation, when it often gives the appearance of two separate, longitudinally split chromosomes, separated by a considerable space, and only connected by two delicate strands (Fig. 19, *a*). That this pair represents the large bivalent is fully established by later stages, in which all intermediate conditions connect it with the large bivalent of the metaphase figure (Fig. 19). The same is certainly true in some cases also of the small bivalent (Fig. 20); but I am not sure of the constancy of this. These facts raise the question whether a very late end-to-end conjugation may not take place, in a manner analogous to that described by Gross ('04) at an earlier period in *Syromastes*. The investigation of this point in smear-preparations will probably yield interesting results.

We now return to the history of the chromatoid body. No sign of it is seen in the spermatogonia, nor can it be positively identified in the spermatocytes until some time after the synizesis. But already in the stages immediately following synizesis, as the chromosome-threads are beginning to spread through the nuclear cavity, from one to three very small, deeply staining granules make their appearance in the protoplasm, usually not far from the nucleus, each of which may often be seen to lie in a clear, vacuole-like space (Fig. 26). In slightly later stages *one* of these granules rapidly enlarges and may soon be distinguished as the chromatoid body (Fig. 27). The other granules seem in many cases to disappear, though they may often be distinguished in addition to the large chromatoid body up to a late stage and even during the divisions (Figs. 29, 30, 10). No evidence can be found that any of these granules are extruded from the nucleus (as has been suggested for the "chromatoid Nebenkörper" by several earlier observers); and it may be pointed out that throughout all these stages both kinds of nucleoli are always clearly visible within the nucleus. Neither can I find evidence of any connection between them and the centrioles, which are first seen in much later stages, lying close against the nuclear membrane.

The chromatoid body reaches its maximum size in Stage *f* or the "confused period" (Fig. 30) when it becomes a most conspicuous element which from this time forward undergoes little or no change until long after its delivery to the spermatids. During this whole period it is always surrounded by a clear, vacuole-like space, and may thus be identified at every stage, even during the divisions. The examination of hundreds of cells during this whole period in *P. senilis* has shown only two or three cases in which more than one such large chromatoid body is present. Its single character seems therefore to be typical of this species. In *P. juniperina*, on the other hand, two such bodies, equal or unequal in size, are more often seen.

From the early post-synaptic stage forwards the nuclei always contain a well defined, conspicuous plasmasome, and one or two intensely stained chromosome-nucleoli. When single, the chromosome-nucleolus is about twice the size shown when two are present (Figs. 26–30). In the earlier stages these bodies are often more or less irregular in form, or elongated. In later stages they are nearly spheroidal, and appear exactly similar, but for their position, to the extra-nuclear chromatoid body. During the prophases the plasmasome disappears, while, as already stated, the chromosome-nucleoli are converted into chromosomes of looser texture, somewhat elongated, and like the bivalents conspicuously split lengthwise. In the final prophases all the chromosomes become condensed and stain intensely, while the chromatoid body is unchanged (Figs. 32–34). Upon dissolution of the nuclear membrane the latter is left nearly in its original position, often close beside the chromosomes, but still always distinguishable from them by the surrounding vacuole. In the anaphases it usually lies rather close to the spindle, often directly upon it (Fig. 10), sometimes actually embedded within it (Figs. 8, 9) as may be proved by careful focusing. It may however lie quite outside the spindle, even near the cell-periphery. In any case it passes bodily into one of the secondary spermatocytes. There are of course two classes of the latter, with and without the chromatoid body (Figs. 22–25); in the former class its history in the second division repeats that seen in the first. The result is that it enters but one fourth of the spermatids, where it lies in the protoplasm outside the nucleus, and owing to its undiminished staining capacity long remains the most conspicuous object in the cell.

The structure and history of the spermatids agrees in the main with the descriptions of Henking, Paulmier, Gross and Montgomery for other Hemiptera, and need not be described in detail. Each spermatid contains besides the nucleus a large, spheroidal chondriosome-body or nebenkern, a much smaller pale sphere from which arises the acrosome (Figs. 36, 37) and (after the earlier stages) a rather large, intensely staining centriole lying close against the nuclear membrane, from which the axial filament grows out (Figs. 38–43). The later history of these structures agrees closely with Montgomery's account of *Euschistus* ('11). In the earlier stages the chromatoid body, when present, may lie at any point, more commonly in front of or behind the nucleus. Whatever be its original position it is sooner or later, without exception, carried far out into the outgrowing tail of the sperm-cell. It may often still be seen near the anterior pole of the spermatid, near the acrosome-sphere, after the latter has performed its first migration to the anterior pole (Fig. 39) but may lie near the opposite pole (Fig. 40). When the sphere again moves backwards towards the posterior pole of the nucleus the chromatoid body moves with it (Fig. 41) and is never again seen near the anterior pole. By the time the acrosome-sphere has again moved forward to its definitive position at the anterior pole of the nucleus the chromatoid body is always in the tail (sometimes much earlier), often at a considerable distance from the head.

As the elongation of the tails proceeds, the chromatoid body is carried still further away from the head, finally reaching a position in the middle tail-region. When the nuclei have become elongate, homogeneous and intensely staining, and the immature spermatozoa are aggregated in parallel bundles, the chromatoid bodies are still conspicuously seen (particularly well in the safranin-green preparations) scattered irregularly within the bundles of sperm-tails. They are at this time still enclosed within the tails, lying in the protoplasm outside the chondriosome-envelope of the axial filament; but their elimination shortly takes place. This process is preceded by a marked accumulation of protoplasm that forms a swelling at one side of the tail within which the chromatoid body lies (Figs. 44, 45); but similar swellings are also seen in the spermatids that contain no chromatoid bodies. That these protoplasmic masses are sloughed off in both cases is certain from the ensuing and final stage, though in my rather scanty material I have never been able to catch the process in the very act. In the succeeding stages numerous protoplasmic balls are found lying between the tails (usually more or less definitely aggregated near the middle tail-region of the bundle) and quite separate from them (Fig. 46). It may now be seen with perfect clearness that the chromatoid bodies have been cast off with the protoplasmic balls; for they are now never within the tails but are still perfectly evident in many of the free protoplasmic balls. Counts of the latter show that the chromatoid body is present in about one fourth of them. It still stains as intensely as ever, and is often quite unchanged, but in many cases has now assumed a crescentic shape, as if the central cavity had broken through to the exterior at one side (Fig. 45).

These facts seems to admit of no other interpretation than that a considerable mass of protoplasm is sloughed off from each spermatid, and that it carries with it the chromatoid body when present. It is certain that the latter does not contribute in any visible way to the formation of the spermatozoön.[1]

At every period of its history the chromatoid body is often homogeneous in appearance; but not infrequently it shows more or less definite indications of a central cavity. Here and there one may be found in which the cavity is clearly evident; and now and then a definite, sharply stained central granule appears within the cavity (Figs. 21, 43). This granule has only been seen in the dividing spermatocytes and in the spermatids. Since centrioles are also seen in these stages they are evidently not derived from the central granule of the chromatoid body.

Apart from its smaller size, the chromatoid body in the three other species mentioned shows the same general history as in *P. senilis*. In *P. juniperina* it is at its maximum size hardly more than half as large as in *P. senilis* (Figs. 34, 35); it is very often accompanied by a much smaller and paler granule lying close beside it (Fig. 35). In *Podisus crocatus* it is somewhat smaller than in *P. juniperina* (Fig. 33), in *Cœnus delius* still smaller and might readily be mistaken for an accidental granule. In all these cases its behavior seems to be of the same type, and it is surrounded by a similar vacuole. In the two forms last mentioned, perhaps because of its small size, its position in the divisions is more variable, and it more often lies away from the spindle or near the periphery of the cell.

II.

The nature of the chromatoid body need not here be considered *in extenso*. As before stated, it is probably of the same nature as the "chromatoid Nebenkörper" described by various observers in other animals; but I know of no case where the facts are in all respects identical with those seen in *Pentatoma*. In the rat, for example, the "chromatoid Nebenkörper" as described by v. Lenhossék ('98) is in some respect remarkably like that of *Pentatoma:* but this body (two are often present in the earlier

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[1] The elimination of protoplasm from the spermatid is of course a well-known and widespread phenomenon. In the cockroach, as described by Morse ('09) the process appears to be similar to that seen in *Pentatoma*, and here also a deeply staining body is cast off, which Morse identifies as a plasmasome. I think it possible, however, that this too may be a chromatoid body comparable with that of *Pentatoma*. I also think it probable that the bodies that have been described as "degenerating cells" in the late spermatid-cysts by some observers are identical with the protoplasmic balls here described.

stages) is stated to be present in all the secondary spermatocytes and also in all the spermatids, where its maximum size is attained. Duesberg ('08) found it only after the first division and believed "qu'il persiste pendant la mitose et se divise pour son propre compte," though the division was not actually seen. Both observers agreed that it degenerates in the spermatid (*cf.* also Meves, '99). Regaud ('10) again describes it in the rat, but finds that it fragments into small granules during the first division and is afterwards reconstituted to form a single body which passes to one pole in the second division, beyond which stage its history was not traced. Such a process of fragmentation certainly does not take place in *Pentatoma*, nor can there be the least doubt of its absence from the greater number of spermatids.

I suspect that the body described by King ('07) as an "acrosome" in the spermatogenesis of *Bufo* belongs in the same general category, though it is described as having a very different history from that seen in either the insects or the mammals. Lastly, I may point out the probable identity of the chromatoid body in *Pentatoma* with that described by Doncaster ('10) in the gall-fly *Neuroterus*, which likewise passes to one pole in the spermatocyte-division. This observer tentatively suggests a possible connection between this body and sex-determination; but the facts seen in the Hemiptera evidently lend no support to this. In none of these cases is the real nature of this body yet clear. In *Pentatoma* it is obviously not a centrosome, centriole, acrosome or extruded nucleolus. Since no definite idiozome is seen in the spermatocytes I suspected for a time that it might be such a body; but this too seems to be excluded by the conditions described in the mammals, where an idiozome is also present.

The nature of the chromatoid body thus remains problematical, but the facts are worthy of serious attention for another reason. Were the chromosomes very small, numerous, closely crowded, or otherwise unfavorable for exact study, and could not the entire history of the chromatoid body be so clearly traced, even an experienced observer might fall into the most confusing error concerning the relations of the chromosomes. It may seem superfluous to urge the danger of confusing with chromosomes other compact and deeply staining bodies that may lie near or among them, or at the spindle poles—extruded nucleoli or nucleolar fragments, chromatoid bodies, "acrosomes," yolk-granules, or the like—but one can not avoid the suspicion that some of the existing contradictions in the literature may have arisen from some such source. Many cases might be cited in illustration of the danger of such confusion. I suggest, for instance, a comparison of my Figs. 9, 10, 22, 23 of *Pentatoma* (first and second division) with Stevens's Fig. 5 of the first division of *Ceuthophilus* ('12), the same author's Fig. 71 of *Stenopelmatus* ('05), Morse's Fig. 46 of *Periplaneta* ('09), and Duesberg's Fig. 45 of the rat ('08). In all of these cases a compact, more or less deeply stained, spheroidal body is seen near one pole in the telophases or late anaphases, lying near the chromosome-group; and the similarity is increased by the fact that in the first three of these cases this body is surrounded by a clear, vacuole-like space. So deceptive is this resemblance that any observer without careful study might readily conclude that the body in question is in each case an accessory chromosome; yet in only two of the five cases would this conclusion be correct. In *Pentatoma* and the rat this body is of protoplasmic origin (chromatoid body), in *Periplaneta*, according to Morse, an extruded nucleolus (plasmasome). Only in *Ceuthophilus* and *Stenopelmatus*, if Miss Stevens's conclusions were correct (I have no reason to doubt that they were) is this body an accessory chromosome.

Such facts make it clear that the presence of sex-chromosomes can not safely be inferred alone from the presence of chromosome-like bodies lagging on the spermatocyte-spindles, or lying near one pole. The presence of compact, deeply staining nucleoli during the growth-period is by itself equally indecisive. In some cases the "plasmasome," especially after certain fixatives such as Bouin's fluid, may stain quite as intensely as the chromosome-nucleoli with hæmatoxylin, safranin and other dyes (*cf.* Gutherz, '12). Decisive evidence regarding these bodies can only be obtained by tracing their individual history and by accurate correlation of the chromosome-numbers in the spermatogonial and spermatocyte-divisions. It hardly need be added that great caution is necessary in dealing with difficult material in which for any reason such a test can not be completely carried out.

Columbia University, February 19, 1913.

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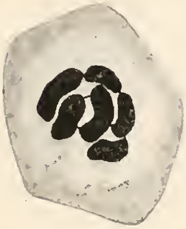
Explanation of Plates.

Plate I.[1]

(*Pentatoma senilis.*)

Figs. 1–3. Spermatogonial metaphase-groups; 4–6, first spermatocyte-division in polar view; 7, early anaphase, the large bivalent also shown separately at the left; 8–10, late anaphases; 11, 12 the same in polar view, sister-groups from the same spindle (the identification of X and Y not entirely certain); 13, second spermatocyte metaphase-group, with chromatoid body; 14–15, the same in side view, chromatoid body absent; 16, the same, with chromatoid body near one pole.

[1] All the figures from camera drawings, enlarged about 2,500 diameters. A designates the acrosome, B the large bivalent, b the small bivalent (or their products), C the chromatoid body, N the Nebenkern or chondriosome-body, X and Y the sex-chromosomes.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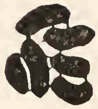
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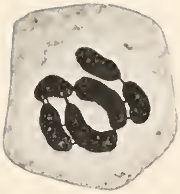
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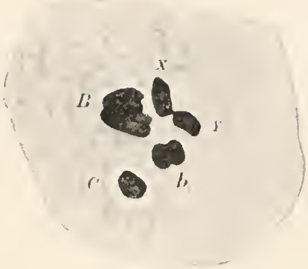

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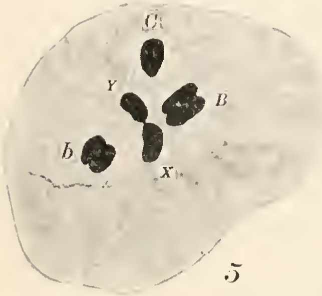

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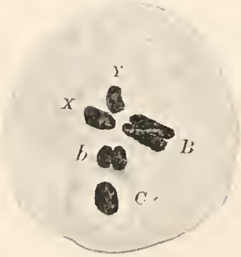

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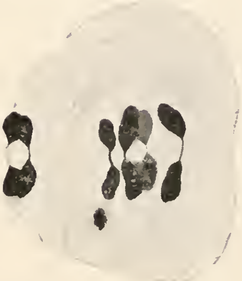
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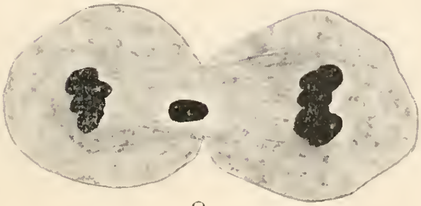
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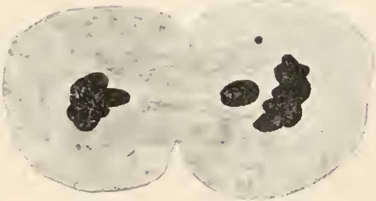
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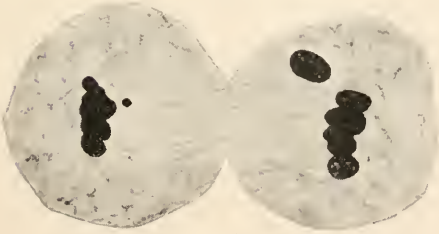
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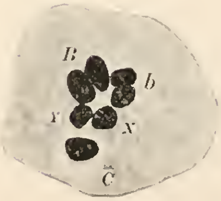

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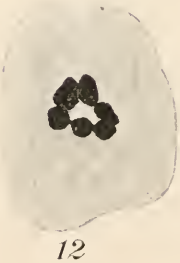
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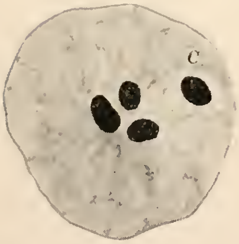

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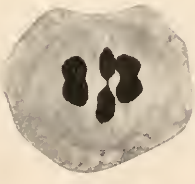
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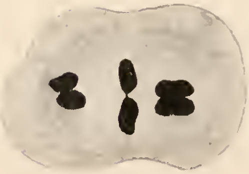
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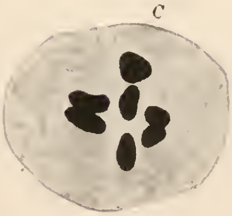

16

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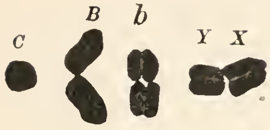
PLATE II.

(*Pentatoma senilis*, except 25.)

FIG. 17. First spermatocyte-metaphase chromosomes from a single spindle, with chromatoid body, artificially spread out in a series for comparison, the large bivalent seen sidewise; 18, *a–f*, the *XY*-pair in successive stages, all in the same position with reference to the spindle (as in Fig. 17), *a* and *b* from metaphases, *c–f* from anaphases; 19, *a–e*, the large bivalent in successive stages, *a–d* from prophase-nuclei, *e* from metaphase; 20, *a–e*, corresponding series of small bivalent, *a–c* from prophase-nuclei, *d*, *e*, from metaphases; 21, tangential section of first division showing *XY*-pair and chromatoid body; 22, 23, second spermatocyte-telophases, with chromatoid body; 24, the same without chromatoid body; 25, the same with chromatoid body near one pole, *Pentatoma juniperina;* 26–29, early growth-period; 30, confused period; 31, middle prophase; 32, late prophase, all the chromosomes foreshortened.



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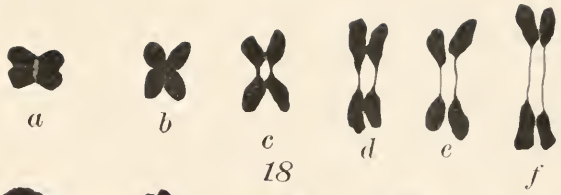

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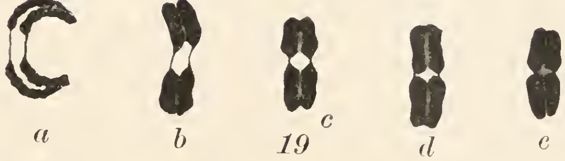

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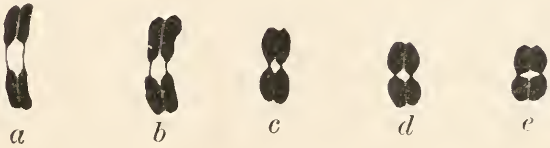

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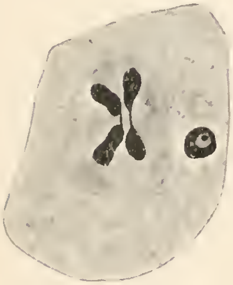

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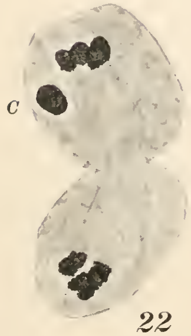

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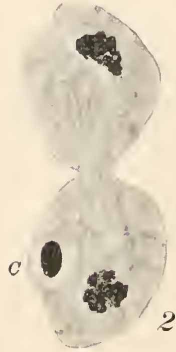

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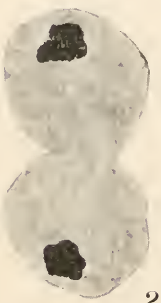

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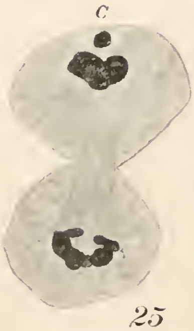

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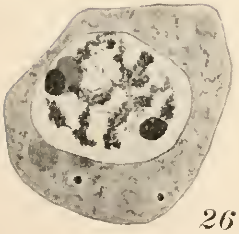

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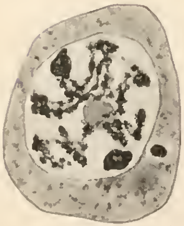

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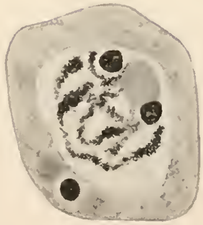

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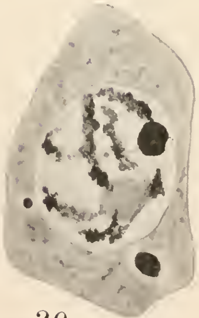

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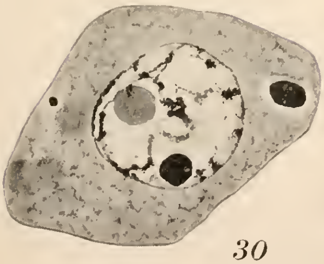

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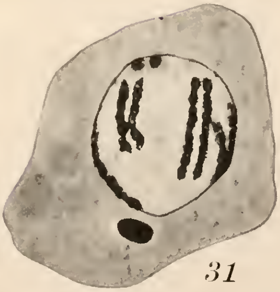

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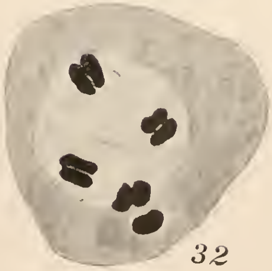

32

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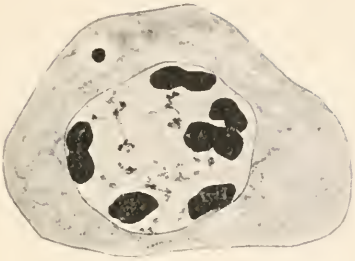
Plate III.

(*Podisus crocatus*, 33; *Pentatoma juniperina*, 34, 35; *P. senilis*, 36–46.)

Fig. 33. Late prophase, *Podisus crocatus*; 34, the same, *Pentatoma juniperina*; 35, first spermatocyte-anaphase of the same species; 36–43, stages in the development of the spermatids, *P. senilis*; the large dark body is the chromatoid body; 44, 45, central region of the tail, later spermatids, showing protoplasmic swelling enclosing chromatoid body; 46, group of cast-off protoplasmic balls lying between the sperm-tails, four containing chromatoid bodies, young spermatozoa.



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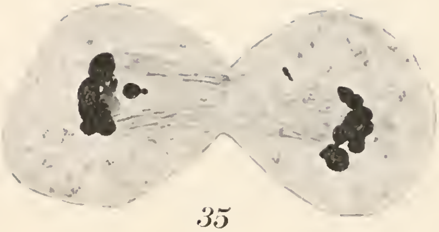

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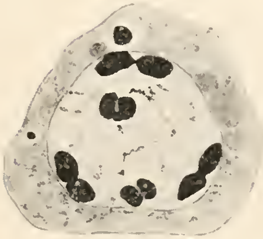

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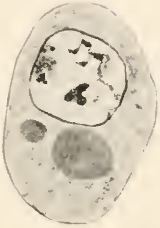

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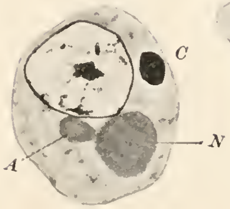

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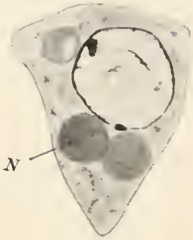

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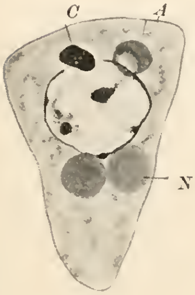

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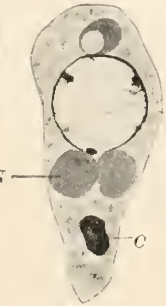

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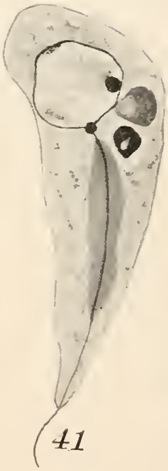

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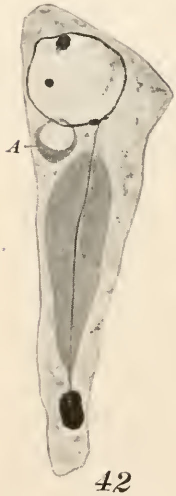

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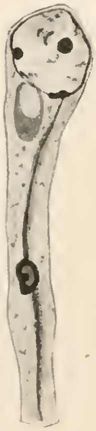

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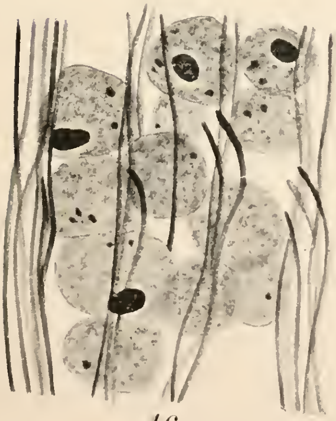

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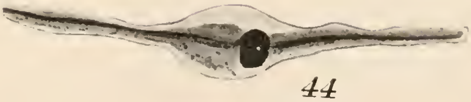

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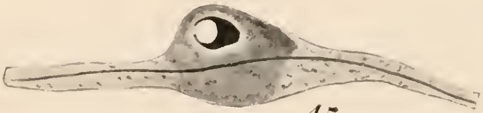


45

E. B. WILSON.

REACTIONS OF AMŒBA PROTEUS TO FOOD.

WILLIAM A. KEPNER AND WILLIAM H. TALIAFERRO,
University of Virginia.

In material taken from a pond southwest of the university we found great numbers of *Amœba proteus*. These animals appeared in a brown scum at the surface of the aquarium. The *Amœba* is one of the simplest animals and hence is an attractive form in which to study the phenomena of life. The simplest vital phenomenon—movement—is of course involved in the food reactions of *Amœba*. Rhumbler and others once held that the movement of *Amœba* could be explained by means of variation of surface tension upon the body of the animal. Jennings was the first to attack this theory. He demonstrated the currents of the protoplasm on the surface of *Amœba* by causing soot to adhere to the surface of the animal. These experiments showed that surface tension could not explain these currents and therefore could not explain the movement of *Amœba*. Later Dellinger ('06) likewise showed that surface tension could not explain this movement, for he saw that in advancing an *Amœba* threw out a pseudopodium, the end of which it fixed by adhesion to the substratum and then through the contraction of this fixed pseudopodium the body was dragged to the point of attachment to the substratum. Rhumbler ('05) according to his reviewer in answer to Jennings' criticism pointed out that in his work "it (Rhumbler's surface tension theory of movement) is not dependent on the movements termed 'Föntanen-strömung,' whose existence Jennings calls in question. These movements, though not frequent, certainly do occur in some *Amœbæ:* it is not their unconditional necessity, but their theoretical value as a starting point, which accounts for their occupying the chief place in the author's theories. It is not claimed that there is more than a parallel value or 'convergence' shown in the comparative experiments with organic and inorganic mechanics; the chemistry in both is fundamentally different. It is possible that in the *Amœba* resides a 'Miniaturpsyche'—an energy absent in the inorganic." Thus the simplest phenomenon of life in one of the simplest animals has not been reduced to terms of physics and chemistry.

Despite this fact there is much, as yet, said concerning the physical and chemical explanation of the food-reactions of *Amœba*. For instance Hegner ('10) says in speaking of the food reaction of *Amœba* that "this apparent choice of food may be due to ordinary physical laws of fluids." Likewise Calkins ('09) says: "While most of the actions of protozoa are reactions to external stimuli, many are combinations of reactions which do not lend themselves to analysis. Such, for example, is the apparent choice of food or of building material for shells and tests, or the complex reactions that are frequently involved in the avoidance of some obstruction. Many of these so-called conscious acts can be explained by the ordinary physical laws of fluids."

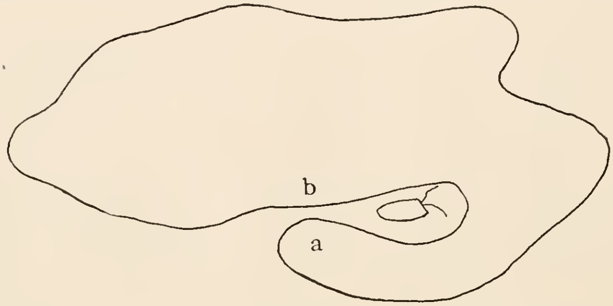
At a more recent date McClendon ('12) showed that by a disturbance of the electrical polarization the surface tension would be modified and adds: "We might conclude therefore that the low surface tension of the *Amœba* is caused by electric polarization, due to the production of some metabolic electrolyte whose anions cannot escape; and that strong stimulation causes increased permeability and hence disappearance of the electrical polarization.

"This would explain all negative tropisms of the *Amœba*. The surface tension of the portion most strongly stimulated is increased, and the *Amœba* flows away from the stimulus.

"In order to explain positive tropisms we would have to make another assumption. If the stimulus did not react directly on the plasma membrane, but penetrated the *Amœba* and acted on the protoplasm, and increased the production of the metabolic product producing polarization of the plasma membrane, it would thereby decrease the surface tension. The local decrease in surface tension would cause the *Amœba* to flow toward the source of the stimulus, just as the quicksilver drop in dilute HNO_3 flows toward potassium bichromate in Bernstein's experiment."

The food-reaction of *Amœba* involves movement. It is however, a more complex phenomenon than mere movement. "The capture and ingestion of food, in its simplest form, occurs in the group of Rhizopoda, where, as in *Amœba proteus*, any part of the body can act as a mouth. In this form pseudopodia are pushed out toward the victim (a flagellate, ciliate, minute plant form of any kind, or even a higher animal, such as a rotifer or worm) and entirely surround it, together with a certain amount of water, thus forming a gastric vacuole, or an improvised 'stomach'" (Calkins '01). This description gives the details as generally given for the ingestion of food.

So far as we have been able to determine no departure from this simple method of capturing food by an *Amœba proteus* has been described. Jennings observed an *Amœba* persistently push a spheroidal *Euglena* cyst from place to place in an effort to ingest it. He also observed an *Amœba proteus* ingest repeatedly a smaller specimen of *Amœba proteus*, the latter each time breaking through the protoplasmic wall of its captor. But even in these most interesting instances the prey was taken into the body by the ingesting protoplasm flowing about each side of the prey with equal velocity. On September 25, Mr. F. L. Kline, Mr. W. A. Williams, Mr. J. P. Williams and Mr. R. T. Scott called the attention of one of us to an *Amœba proteus* that was sending a pseudopodium out from the side of its body posteriorly in such a way as to surround a quiet *Chilomonas paramœcium*. In this case it was remarkable that the entrapping protoplasm was flowing about but one side of the prey, the side of the *Amœba's* body furnishing one wall of the forming food vacuole. Later Mr. Scott observed that the food vacuole was completed through the fusion of the end of the pseudopodium and the side of the body (text-figure 1). September 26, Mr. E. M. Baker called our attention to an *Amœba proteus* that had a *Chilomonas paramœcium* lying between two pseudopodia as indicated in Fig. 1, *a*. He had not observed where the contact with the prey had been made, but it was evident that the two pseudopodia were closing up behind the *Chilomonas paramœcium*. We then called the attention of Mr. C. A. Amos, Mr. H. H. Buehler, and Mr. A. H. Brewster to the specimen. As we took turns with these men in observing it the left pseudopodium alone grew. As it elongated it travelled along the inner side of the right pseudopodium to its base. At the base of the right pseudopodium the growing left one turned along the surface of the body and then travelled "anteriorly" until the prey was entwined by it. When thus the enclosing space had been greatly reduced the left pseudopodium fused along its two margins at the place indicated in Fig. 1, *e* by a broken line.



TEXT-FIGURE 1. Outline of an *Amœba proteus*, showing the manner in which the pseudopodium *a* developed along the outer side of a *Chilomonas*. Eventually the apex of the developing pseudopodium, *a*, fused with the body at *b*. In this manner the *Chilomonas* was taken into the body of the *Amœba*. (The position of the axis of the *Chilomonas* was not observed. The point of contact of the *Chilomonas* with the surface of the *Amœba* was likewise not noted.)

These observations led us to make further studies on the food reactions of *Amœba proteus*. An *Amœba proteus* with two pseudopodia projecting and flowing for the most part into the larger one came in contact with a quiet *Chilomonas paramœcium* (Fig. 2). The *Chilomonas paramœcium*, thus disturbed, retreated to the position indicated by the finely stippled outline (Fig. 2). Again the pseudopodium came in contact with the *Chilomonas* and the latter then retreated to the position indicated by the darkest contour. As the *Chilomonas* lay in this position the *Amœba proteus* approached it and this time without disturbing the flagellate sent protoplasmic processes about its sides until the prey was enclosed within a food vacuole. This observation does not depart greatly from what has been generally described as the usual reaction of *Amœba proteus* to food.

The next observation, however, is quite unusual. The *Chilomonas paramœcium* in this case ran into the side of an *Amœba proteus* that was "flowing into" a curved pseudopodium. In this case the large pseudopodium flowed back along the outer side of the *Chilomonas* until the tips of the two pseudopodia fused (Fig. 3).

We have seen three instances of a *Chilomonas paramœcium* and one instance of a diatom entering the *narrow* angle between two pseudopodia and coming in contact with the ectoplasm at the apex of this angle. In all these cases the reactions were analogous. The specimen represented in Fig. 4 had a *Chilomonas paramœcium* swim into the narrow angle between two pseudopodia and which made repeated contacts at *a*. The response to this stimulus at *a* resulted in the formation of pseudopodia behind the *Chilomonas* at *b* and *c*.

In contrast with the last observations is one made upon a specimen that had two *widely* diverging pseudopodia. The general movement of the body was in the larger pseudopodium. A *Chilomonas paramœcium* came in contact with the middle of the mesial surface of the smaller pseudopodium. The flagellate made a single impact at this point and lay in contact with the ectoplasm. In response to this contact the *Amœba proteus* sent out a third protoplasmic process from the apex of the angle between the first two pseudopodia (Fig. 5, *a*), thus placing the object of prey in a narrow angle between two pseudopodia. Before the end of pseudopodium *a* reached the level of the end of its neighbor it changed its course so as to flow behind the *Chilomonas*. At the same time the original pseudopodium sent out a secondary one (Fig. 5, *b*) below its apex to meet the other enclosing pseudopodium. In this way the *Chilomonas* was enclosed in a food vacuole of about the usual size.

Other conditions may arise with reference to the capturing of the *Chilomonas paramœcium* by the *Amœba proteus*. *Chilomonas* sometimes seems quite indifferent to the contact of the *Amœba proteus*. On September 30, a specimen of the latter was observed which came in contact with a flagellate at the apex of its more active pseudopodium (Fig. 6, *a*, 1.) As the pseudopodium grew the *Chilomonas* glided along its surface to the position 2 in Fig. 6, *a*. When the flagellate was left a little farther beyond the apex of the growing pseudopodium the streaming movement of the latter changed its course so as to cause the pseudopodium to send a process or secondary pseudopodium off behind the quiet *Chilomonas*. This process or secondary pseudopodium, through a streaming at right angles to the larger one, grew somewhat larger, then its course became oblique and it traveled like a wave posteriorly along the mesial surface of its parent pseudopodium. This wave-like movement of the secondary pseudopodium carried the yet quiet *Chilomonas* down to the apex of the angle between the two original pseudopodia. Thus the prey was brought into a relatively small space, which through the fusion of the tip of the secondary pseudopodium and a region near the base of one of the original pseudopodia, became a food vacuole (Fig. 6, *a*, *b*, *c* and *d*).

Perhaps the most interesting condition we have seen presented to the ingenuity of the *Amœba proteus* is to be seen in the following observation. The *Amœba proteus* was traveling along the line of a large pseudopodium when it came in contact with a quiet *Chilomonas paramœcium*. At first the *Amœba proteus* seemingly did not react but simply pushed the *Chilomonas* to one side (Fig. 7, 2). At this point, however, the *Amœba proteus* protruded a small pseudopodium from the apex of the larger one which proceeded around the *Chilomonas* (Fig. 7, *a*). At the same time it threw out a secondary pseudopodium from the body of the larger one some distance below the *Chilomonas* (Fig. 7, *b*). It, however, withdrew this secondary pseudopodium at *b* and threw out another one (Fig. 7, *c*), closer to the *Chilomonas* which immediately proceeded to meet the first enclosing pseudopodium, *a*.

After the extreme variability of the *Amœba's* reactions to food as seen in these observations one is not justified in saying as does Hegner ('10), "This apparent choice of food may be due to the ordinary physical laws of fluids." In the first place as many if not more *Chilomonas paramœcia* were rejected as were accepted by the *Amœbas*. Frequently a *Chilomonas paramœcium* would come in contact with the "anterior end" or side and then remain by the *Amœba* without being ingested. If this were a process involving the "ordinary physical laws of fluids" there would be no rejection of food when it presented itself in a manner favorable for acceptance. Nor does it follow that when an *Amœba* has rejected food that this rejection is final. On September 26, Mr. L. Grady Burton saw an *Amœba proteus* which was flowing along the line of a large pseudopodium come in contact with a quiet *Chilomonas paramœcium*. The *Amœba proteus* twice touched the *Chilomonas* and each time withdrew its pseudopodium. The *Amœba proteus* then moved around and by the *Chilomonas*. It now lay opposite a side which was at right angles to the one first encountered. The *Amœba* then sent out a pseudopodium at right angles to the first one and again touched the *Chilomonas*. It rejected the flagellate again but on touching it the second time in this new position the *Amœba proteus* ingested the *Chilomonas*.

These reactions too show a departure from the description given by Jennings ('06). "The essential features of the food reaction seem to be the movement of the *Amœba* toward the food body (long continued, in some cases), the hollowing out of the anterior end of the *Amœba*, the sending forth of pseudopodia on each side of and above the food, and the fusion of the free ends of the pseudopodia thus enclosing the food, with a quantity of water. The reaction is thus complex; at times, as we have seen, extremely so."

As we have been able to observe the reactions of the *Amœba proteus* are not always "the hollowing out of the anterior end of the *Amœba*, the sending forth of pseudopodia on each side of and above the food, and the fusion of the free ends of the pseudpodia"; but the reaction is a variable one and may arise from the side of the *Amœba's* body and involve primarily but a single pseudopodium.

Moreover the reaction of an *Amœba proteus* to food is in each case determined by the conditions presented at the time of contact. Some of these conditions are:

1. The metabolic condition of the *Amœba proteus*, demanding or not demanding food.

2. The form of the *Amœba's* body at the time the food is presented to it with reference to (*a*) the amount of water that may enter a food vacuole, and (*b*) the possible retreat of the object of prey.

So far as our observations are concerned little can be said about the first condition. The relative number of food vacuoles did not seem to make any marked difference in the conduct of the *Amœba* with reference to food. It was not in our power in any way to determine whether the metabolic conditions of the *Amœba* demanded food or not.

Our observations are chiefly concerned with the manner in which the *Amœba* met the conditions of not getting too great an amount of water into the food vacuole and of not letting the prey escape. We can readily understand how by dilution of ferments a relatively large quantity of water in a food vacuole might present a condition to the *Amœba* in taking in food. The amount of water, therefore, that may enter a food vacuole is one of the dominate factors in controlling the way in which an *Amœba* reacts to the prey.

This condition we infer was the primary one determining the action of the *Amœba* represented in Fig. 1. Here the object of prey when first seen lay out away from the surface in a wide angle between two pseudopodia. The stimulus of its contact (not observed by any of us) resulted in the pseudopodia bending their tips towards each other. Had these tips fused the basis of a very large food vacuole would have been formed. No fusion took place here. In the manner described above the one pseudopodium was wrapped about the *Chilomonas* until a relatively small space was enclosed over which an ectoplasmic film arched leaving only a small bilobed aperture (Fig. 1, *e*, *ap*). Out of this small bilobed aperture water was apparently forced, for the enclosure became gradually smaller and after it was reduced to the size of the food vacuole usually encountered the bilobed opening was closed through the convergence and fusion of its lips. After this pore was closed the endoderm also came to lie over the upper side of the food vacuole. In this rather remarkable manner the prey was not allowed to escape and a food vacuole with a relatively small amount of water was formed.

The reaction of the *Amœba* represented in Fig. 2 is like that usually described for the conduct of an *Amœba* with reference to food. Here the amount of water that is to enter the food vacuole is not so great a conditioning factor but the reaction is rather conditioned by the great chances afforded for the escape of the prey. So after disturbing the prey twice and causing it to retreat as often along the projected axis of the pseudopodium the *Amœba* did not disturb the prey but sent about it pseudopodia which eventually enclosed the quiet *Chilomonas*. This is of further interest in demonstrating that the reaction of *Amœba* to food is not a fixed one for twice the *Chilomonas* was rejected and the third time deliberately accepted.

In contrast with this reaction is that of the *Amœba* shown in Fig. 3. Here a *Chilomonas*, after making contact with the ectoplasm of the *Amœba*, lay in between two pseudopodia, one of which presented an inwardly directed surface and the other an outwardly directed surface. If the disturbed prey were to strike the former it would be deflected towards the fundus of the interpseudopodial space, whereas if the disturbed prey were to strike the latter it would be deflected away from the space which is to form the basis of a food vacuole. Thus in this case the possible retreat of the prey may be the chief condition to be met.

When an object of prey entered a narrow angle between two pseudopodia the possible retreat of the prey again became the principal conditioning factor. If the *Amœba* had reacted at the point of contact by sending out secondary pseudopodia towards each side of the prey it would have crowded back the prey and thus result in its escape. To prevent this escape the *Amœba* sent secondary pseudopodia behind the *Chilomonas paramœcium* as described for Fig. 4 on page 415.

Further it is interesting to note that in this case the stimulus was encountered at *a* and the reaction took place at *b* and *c* (Fig. 4). Thus we see that here it is not as Jennings ('06) says in reference to locomotion that "it is primarily the part stimulated that responds." For in this reaction it was not the part immediately stimulated but the parts that could most advantageously respond that did so.

Where it was more advantageous for another part or parts to respond we find that they responded. Take for example the case in which the object of prey entered a broad angle between two pseudopodia as described on page 415 for Fig. 5. If the *Amœba* had reacted in the manner just described it would have been easier for the prey to escape and the food vacuole would have contained a large quantity of water. For these and probably other unknown reasons the *Amœba* reacted by sending out a secondary pseudopodium which placed the prey in a narrow angle and then ingestion took place practically the same way as described above for the reaction to food in a narrow angle (Fig. 5).

Before leaving observations 4 and 5 a useful comparison should be made. In the reaction of the *Amœba* represented in Fig. 4 the suggestion might be raised that pseudopodia grew with equal velocity at *b* and *c* because of more or less equal stimuli caused by vortices in the wake of the prey. But in answer to this suggestion we have the reaction of the *Amœba* in Fig. 5. Here we see the contact was made obliquely to the surface and that the larger pseudopodium arose at a point most remote from the path of the prey. *So the reaction was greatest where the stimulus was weakest but where the conditions demanded greatest reaction.*

When the *Chilomonas paramœcium* in Fig. 6 was left in position 2 it lay at a point on the surface which would not direct its possible movements towards the fundus of the space between the two pseudopodia. So, as if in order to prevent its direct retreat from this space, a secondary pseudopodium grew up behind the prey. The manner in which the secondary pseudopodium travelled as a wave along the mesial surface towards the fundus of the interpseudopodial space met the conditions determined by the possible escape of the prey and the usual or maximum size of a food vacuole.

The second relatively large pseudopodium in this case seems to have been a determining structure in this reaction. The position of the *Chilomonas* too may have been a factor in it, for in these two respects the *Amœbas* shown in Figs. 6 and 7 differ. In the latter there is no second pseudopodium to coöperate with the first and the axis of the body of the *Chilomonas* shown in Fig. 6 lies more or less parallel to the surface of the pseudopodium whereas in the *Amœba* shown in Fig. 7 the *Chilomonas* in position 2 lies at right angles to the surface of the pseudopodium. Thus the *Chilomonas* in the latter instance has all paths for retreat open except that towards the surface of the pseudopodium. Hence the possibility of escape of the prey again becomes the conditioning factor in the reaction, which is met in the manner described on page 416.

In the *Amœba* which presented the reaction shown in Fig. 7 we again see that it is not the part stimulated that primarily reacts nor is the response for a given stimulus a fixed one. The stimulus in this case was made at the point *d* and at first the tip of the pseudopodium *a* grew along the far side of the prey while a smaller pseudopodium arose at *b*. Later this first reaction was greatly modified to better meet the involved conditions. This modification was accomplished through a negative response at *b* and a positive response at *c*. This reaction, so modified, was a better one in so far that it made the escape of the *Chilomonas* less probable. Thus we have an example of the *Amœba proteus* attaining its end through trial and error since the reaction to a given stimulus was modified with reference to more readily realizing the object of the reaction.

CONCLUSIONS.

1. The reactions of *Amœba proteus* are highly variable in reference to: (*a*) power of accepting or rejecting food; (*b*) method of ingesting food.

2. In all this variability of reaction there appears no haphazardness. The reactions are not automatic responses. Each reaction is a response made to suit the peculiar conditions presented at the moment of accepting or rejecting the food; and if not well suited to meet these conditions, it is modified with reference to better meeting them. Thus in each reaction there is evidence of purposiveness.

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[1] The same day that the page proof of this article came to the authors Prof. F. J. Wright sent us a reprint of "Daily Life of Amœba Proteus" by David Gibbs, Clark University. No reference has been made to this important paper in our paper. Authors.

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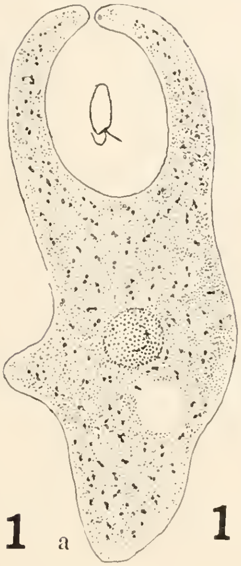
EXPLANATION OF PLATE I.

FIG. 1. (*a*) The pseudopodia have closed behind the prey; (*b*), (*c*), (*d*), and (*e*) shows how the left pseudopodium grew down along and then away from the right pseudopodium. The broken line in (*e*) indicates the place where the two surfaces of the left pseudopodium fused to enclose the prey. (*ap*) indicates the bilobed margin of the ectoplasm that over-arched the forming food vacuole. ×166.

FIG. 2. The *Chilomonas paramœcium* was first encountered at position 1. The prey next retreated to position 2, indicated by the dotted contour. When the second contact with the *Amœba proteus* was made it retreated to position 3. The contour of the position 3 is indicated by the darkest line. It lay in this third position when it was ingested by the *Amœba*. ×333.



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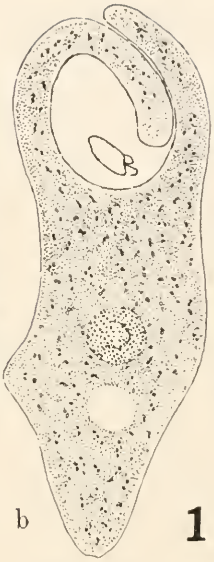

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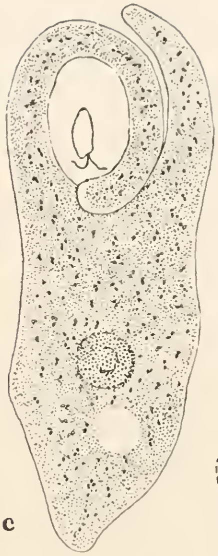

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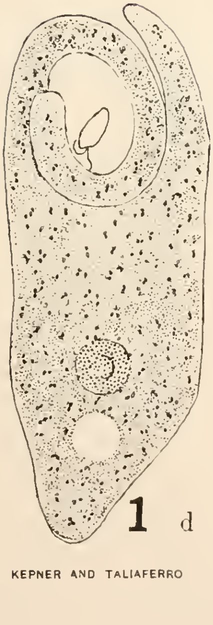

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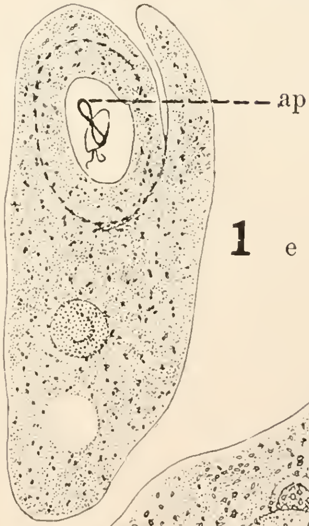

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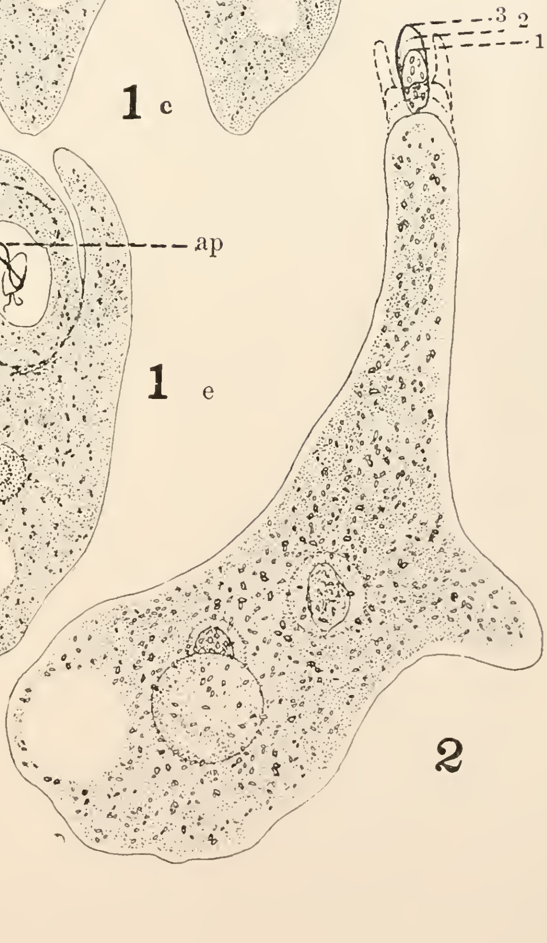

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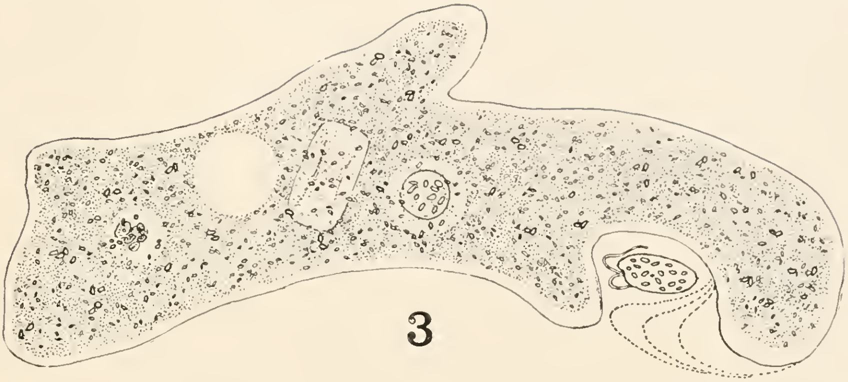
Explanation of Plate II.

Fig. 3. Shows a *Chilomonas* lying between a lateral and a terminal pseudopodium. The response to the contact or contacts made by the *Chilomonas* was the lateral and posterior growth of only the terminal pseudopodium. The successive steps in the advance of this pseudopodium is indicated by the broken lines. ×333.

Fig. 4. The *Chilomonas* by advancing and retreating made three contacts at *a*. The response of the *Amœba* to these contacts was not at the point stimulated but at points (*b*) and (*c*). From these points secondary pseudopodia grew towards each other and fused behind the prey, as indicated by the dotted lines. ×333.

Fig. 5. A *Chilomonas* entered the wide angle between the two large pseudopodia in the direction indicated by the arrow. When it met the *Amœba* it lay in contact with the ectoplasm. In response to this contact a small secondary pseudopodium (*b*) arose behind the prey and a large pseudopodium (*a*) arose from the apex of the inter-pseudopodial angle. ×333.



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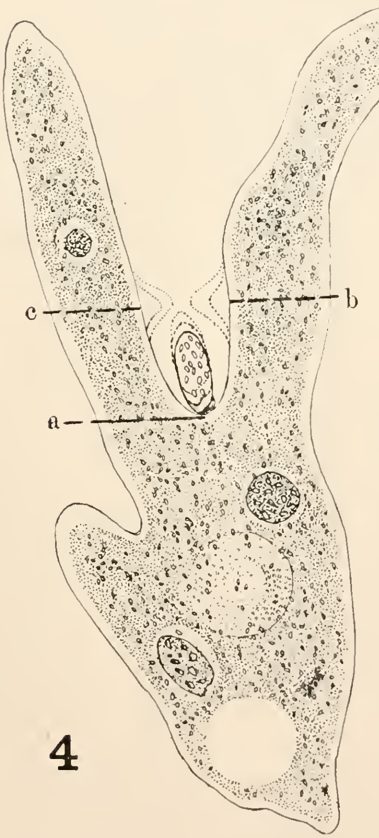

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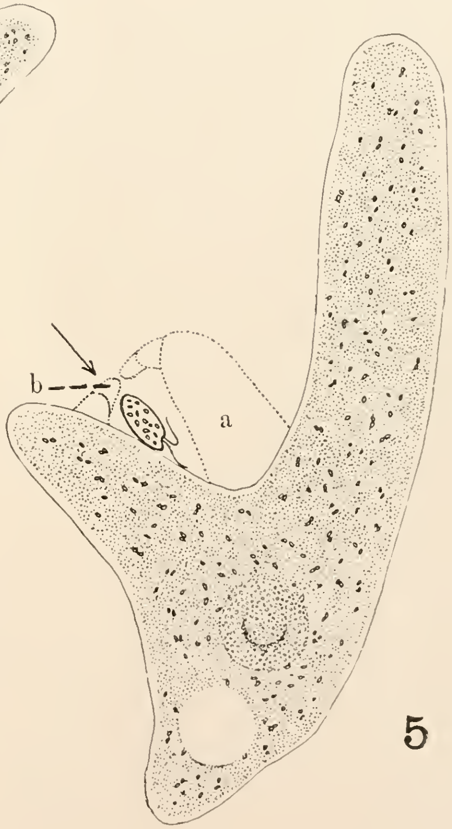

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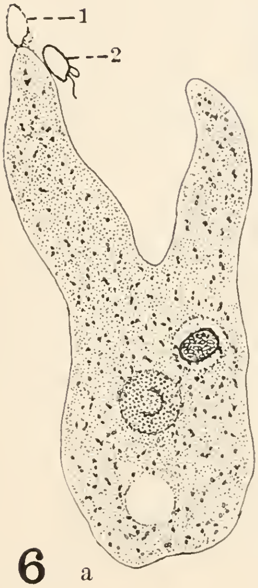
KEPNER AND TALIAFERRO.

EXPLANATION OF PLATE III.

FIG. 6. (*a*) The *Chilomonas* first lay at the apex of the pseudopodium. As the pseudopodium advanced it glided by the *Chilomonas* so that the latter lay to the right in contact with the ectoplasm at the point indicated by position 2. (*b*), (*c*), and (*d*) show the wave-like character of the reaction. This reaction crowded or dragged the passive prey down into the fundus of the interpseudopodial space where the *Chilomonas* was enclosed in a vacuole formed by the fusion of the apex of the lateral active pseudopodium and the base of the right, inactive pseudopodium. ×166.

FIG. 7. The large pseudopodium of *Amœba* came in contact at its apex with the *Chilomonas* (1). The *Chilomonas* was then pushed to position (2). The pseudopodium next advanced beyond the prey and turned to the right at (*a*) along its upper side. At the same time a secondary pseudopodium was thrown out at (*b*). Later this reaction on the part of the *Amœba* was changed; the pseudopodium at (*b*) was withdrawn and one thrown out at (*c*) to better meet the conditions necessary for the capture of the prey. Pseudopodia (*a*) and (*c*) then grew around the *Chilomonas* and enclosed it in a food vacuole. ×333.



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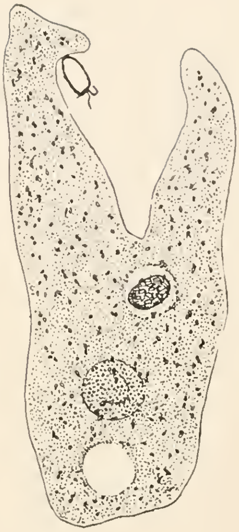

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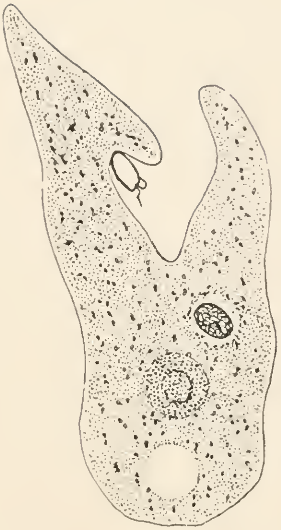

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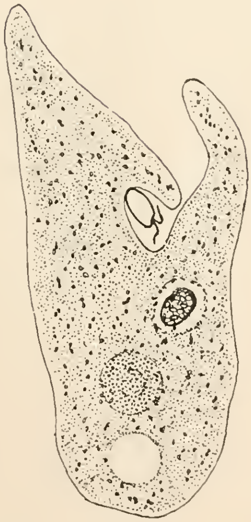

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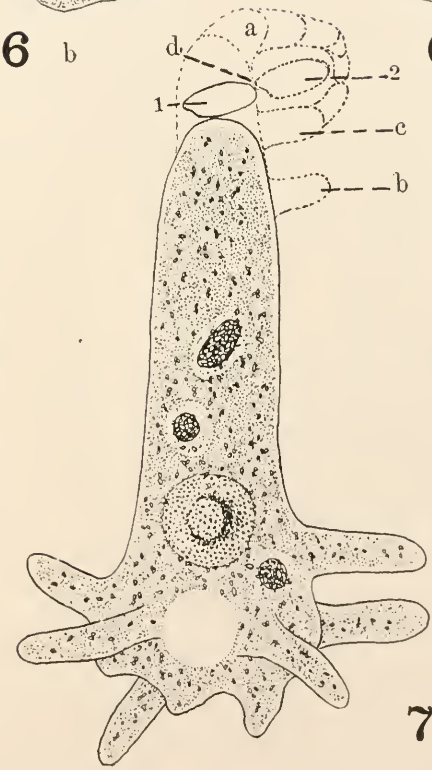

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KEPNER AND TALIAFERRO.

THE MARINE BIOLOGICAL LABORATORY

FIFTEENTH REPORT

FOR THE YEAR 1912

I. LIST OF TRUSTEES 429
II. ACT OF INCORPORATION 431
III. BY-LAWS 432
IV. TREASURER'S REPORT 434
V. THE DIRECTOR'S REPORT 438
  Introduction 439
  1. The Staff—1912 443
  2. Investigators and Students—1912 446
  3. Tabular View of Attendance 451
  4. Subscribing Institutions 452
  5. Evening Lectures—1912 453
  6. Members of the Corporation 454

I. TRUSTEES

EX OFFICIO

F. R. LILLIE, *Director*, The University of Chicago.
GILMAN A. DREW, *Assistant Director*, Marine Biological Laboratory.
D. BLAKELY HOAR, *Treasurer*, 161 Devonshire Street, Boston, Mass.
G. N. CALKINS, *Clerk of the Corporation*, Columbia University.

TO SERVE UNTIL 1916

H. H. DONALDSON	Wistar Institute of Anatomy and Biology.
M. J. GREENMAN	Wistar Institute of Anatomy and Biology.
C. W. HARGITT	Syracuse University.
H. S. JENNINGS	Johns Hopkins University.
GEORGE LEFEVRE	University of Missouri.
A. P. MATHEWS	The University of Chicago.
G. H. PARKER	Harvard University.

TO SERVE UNTIL 1915

H. C. BUMPUS	University of Wisconsin.
R. A. HARPER	Columbia University.
W. A. LOCY	Northwestern University.
JACQUES LOEB	Rockefeller Institute for Medical Research.
F. P. MALL	Johns Hopkins University.
GEORGE T. MOORE	Missouri Botanical Garden, *Secretary of the Board.*

L. L. Nunn.............Telluride, Colo.
John C. Phillips.......299 Berkeley Street, Boston, Mass.

TO SERVE UNTIL 1914

Cornelia M. Clapp.....Mount Holyoke College.
E. G. Conklin.........Princeton University.
Ross G. Harrison......Yale University.
Camillus G. Kidder....27 William Street, New York City.
M. M. Metcalf........Oberlin College.
William Patten........Dartmouth College.
Jacob Reighard.......University of Michigan.
W. B. Scott............Princeton University.

TO SERVE UNTIL 1913

S. F. Clarke...........Williams College.
Charles Coolidge......Ames Building, Boston, Mass.
C. R. Crane...........2559 Michigan Boulevard, Chicago, Ill.,
President of the Board.
Alfred G. Mayer......Carnegie Institution.
T. H. Morgan.........Columbia University.
Erwin F. Smith........United States Department of Agriculture.
E. B. Wilson...........Columbia University.

II. ACT OF INCORPORATION

No. 3170.

COMMONWEALTH OF MASSACHUSETTS

Be It Known, That whereas Alpheus Hyatt, William Sanford Stevens, William T. Sedgwick, Edward G. Gardiner, Susan Minns, Charles Sedgwick Minot, Samuel Wells, William G. Farlow, Anna D. Phillips and B. H. Van Vleck have associated themselves with the intention of forming a Corporation under the name of the Marine Biological Laboratory, for the purpose of establishing and maintaining a laboratory or station for scientific study and investigation, and a school for instruction in biology and natural history, and have complied with the provisions of the statutes of this Commonwealth in such case made and provided, as appears from the certificate of the President, Treasurer, and Trustees of said Corporation, duly approved by the Commissioner of Corporations, and recorded in this office;

Now, therefore, I, HENRY B. PIERCE, Secretary of the Commonwealth of Massachusetts, *do hereby certify* that said A. Hyatt, W. S. Stevens, W. T. Sedgwick, E. G. Gardiner, S. Minns, C. S. Minot, S. Wells, W. G. Farlow, A. D. Phillips, and B. H. Van Vleck, their associates and successors, are legally organized and established as, and are hereby made, an existing Corporation, under the name of the MARINE BIOLOGICAL LABORATORY, with the powers, rights, and privileges, and subject to the limitations, duties, and restrictions, which by law appertain thereto.

Witness my official signature hereunto subscribed, and the seal of the Commonwealth of Massachusetts hereunto affixed, this twentieth day of March, in the year of our LORD ONE THOUSAND, EIGHT HUNDRED and EIGHTY-EIGHT.

HENRY B. PIERCE,
Secretary of the Commonwealth.

[SEAL.]

III. BY-LAWS OF THE CORPORATION OF THE MARINE BIOLOGICAL LABORATORY

I. The annual meeting of the members shall be held on the second Tuesday in August, at the Laboratory, in Woods Hole, Mass., at 12 o'clock noon, in each year, and at such meeting the members shall choose by ballot a Treasurer and a Clerk, who shall be, *ex officio*, members of the Board of Trustees, and Trustees as hereinafter provided. At the annual meeting to be held in 1897, not more than twenty-four Trustees shall be chosen, who shall be divided into four classes, to serve one, two, three, and four years, respectively, and thereafter not more than eight Trustees shall be chosen annually for the term of four years. These officers shall hold their respective offices until others are chosen and qualified in their stead. The Director and Assistant Director, who shall be chosen by the Trustees, shall also be Trustees, *ex officio*.

II. Special meetings of the members may be called by the Trustees, to be held in Boston or in Woods Hole at such time and place as may be designated.

III. The Clerk shall give notice of meetings of the members by publication in some daily newspaper published in Boston at least fifteen days before such meeting, and in case of a special meeting the notice shall state the purpose for which it is called.

IV. Twenty-five members shall constitute a quorum at any meeting.

V. The Trustees shall have the control and management of the affairs of the Corporation; they shall present a report of its condition at every annual meeting; they shall elect one of their number President and may choose such other officers and agents as they may think best; they may fix the compensation and define the duties of all the officers and agents; and may remove them, or any of them, except those chosen by the members, at any time; they may fill vacancies occurring in any manner in their own number or in any of the offices. They shall from time to time elect members to the Corporation upon such terms and conditions as they may think best.

VI. Meetings of the Trustees shall be called by the President, or by any two Trustees, and the Secretary shall give notice thereof by written or printed notice sent to each Trustee by mail, postpaid.

Seven Trustees shall constitute a quorum for the transaction of business. The Board of Trustees shall have power to choose an Executive Committee from their own number, and to delegate to such Committee such of their own powers as they may deem expedient.

VII. The President shall annually appoint two Trustees, who shall constitute a committee on finance, to examine from time to time the books and accounts of the Treasurer, and to audit his accounts at the close of the year. No investments of the funds of the Corporation shall be made by the Treasurer except approved by the finance committee in writing.

VIII. The consent of every Trustee shall be necessary to a dissolution of the Marine Biological Laboratory. In case of dissolution, the property shall be given to the Boston Society of Natural History, or some similar public institution, on such terms as may then be agreed upon.

IX. These By-Laws may be altered at any meeting of the Trustees, provided that the notice of such meeting shall state that an alteration of the By-Laws will be acted upon.

X. Any member in good standing may vote at any meeting, either in person or by proxy duly executed.

IV. TREASURER'S REPORT

For the Year Ending December 28, 1912

Receipts

Annual Dues	$ 716.00	
Chemicals	7.83	
Charles R. Crane	16,500.00	
Fire insurance (Botany Building)	995.19	
Mess	10,088.12	
Mess extension	1,007.47	
Miscellaneous:		
Interest on deposits, use of drain, rent of microscopes, telephones, notary fees, etc.	91.48	
Rockefeller Institute	300.00	
Supply Department	13,966.35	
Tuitions	4,875.00	$48,547.44

Payments

Overdraft on December 22, 1911	$ 56.41
Adminstration (including expenses of treasurer's office)	5,696.29
Bath house	40.92
Biological Bulletin (net)	951.05
Boats	4,452.97
Carpenter	299.93
Chemical Department	1,351.66
Dormitories	385.41
Fish trap	1,510.02
Homestead, ice house	206.18
Homestead, shop	12.30
Homestead, mess	8,507.02
Instructors' salaries	3,125.00
Insurance	4.64
Interest	150.00

Library	1,243.09	
Machinery and tools	199.46	
Maintenance of buildings and grounds	2,100.09	
Mess extension	1,749.04	
New laboratory	42.00	
Oil house	49.26	
Pile driver	119.45	
Printing and advertising	40.86	
Real estate	801.00	
Repairs to botany building	187.06	
Scientific apparatus	277.36	
Stone building	7.00	
Store house	896.90	
Sundries	388.72	
Supply department	8,907.65	$43,758.74
Cash on hand December 28, 1912		$ 4,788.70

MARINE BIOLOGICAL LABORATORY INVESTMENTS

JANUARY 1, 1913

RESERVE FUND

Amount of fund December 1, 1899	$4,553.14	
Received from life memberships	600.00	
Income to January 1, 1913	2,516.90	
Gain from sale of securities and rights	372.38	
	8,042.42	
Paid for current expenses of Laboratory	6,000.	$2,042.42
Reserve Fund now consists of the following:		
$3,000 Am. Tel. & Tel. Co. 4s cost	$2,921.25	
6 shs. Am. Smelting & Refining Co. Pfd. cost	732.00	
5 shs. Gen. Electric Co.	756.25	
14 shs. United Shoe Mach. Corp. Pfd. cost	393.75	
Cash	239.17	
	$5,042.42	
Part of the above stocks and bonds are held as collateral for loan of	3,000.	$2,042.42

LIBRARY FUND

Amount of fund December 1, 1899	$ 866.15	
Income to January 1, 1913	866.27	
Gain from sale of securities and rights	96.39	$1,828.81
The Library Fund now consists of the following:		
3 shs. Am. Tel. & Tel. Co. cost	$ 383.25	
4/5 of $1,000 Am. Tel. & Tel. Co. 4s cost	799.00	
1 sh. Am. Smelting & Refining Co. Pfd. cost	122.00	
2 shs. General Electric Co. cost	302.50	
5 shs. United Shoe Mach. Corp. Pfd. cost	140.63	
Cash	101.43	$1,828.81

LUCRETIA CROCKER FUND

Amount of fund December 1, 1899	$2,500.	
Income after paying students' fees	643.41	
Sale of rights	8.79	$3,152.20
The Lucretia Crocker Fund now consists of the following:		
18 shs. Vermont & Mass. R. R. Co. cost	$2,416.50	
1 sh. West End Street R'y Co. cost	83.	
1 sh. Am. Tel. & Tel. Co. cost	127.75	
1/5 sh. of $1,000 Am. Tel. & Tel. Co. 4s cost	194.75	
1 sh. General Electric Co.	151.25	
Cash	178.95	$3,152.20

CASH RECEIPTS AND DISBURSEMENTS ON ACCOUNT OF FUNDS SINCE AUGUST 13, 1912

Reserve Fund

Cash on hand August 13, 1912	$ 202.92	
Div. 6 shs. Am. Smelting & Refining Co. Pfd. Sept. 1	10.50	
Div. 6 shs. Am. Smelting & Refining Co. Pfd. Dec. 1	10.50	
Div. 5 shs. Gen. Elec. Co.	10.	
Div. 14 shs. United Shoe Mach. Corp. Pfd.	5.25	$ 239.17

Crocker Fund

Cash on hand August 13, 1912	$ 119.20	
Div. 1 sh. West End St. Ry.	1.75	
Div. 18 shs. Vermont & Mass. R. R.	54.	
Div. 1 sh. Gen. Elec. Co.	2.	
Div. 1 sh. Am. Tel. & Tel. Co.	2.	178.95

Library Fund

Cash on hand August 13, 1912	$ 86.05	
Div. 1 sh. Am. Smelting & Refining Co. Pfd. Sept. 1	1.75	
Div. 1 sh. Am. Smelting & Refining Co. Pfd. Dec. 1	1.75	
Div. 5 shs. United Shoe Mach. Corp. Pfd.	1.88	
Div. 2 shs. Gen. Electric Co.	4.00	
Div. 3 shs. Am. Tel. & Tel. Co.	6.00	101.43
Cash on hand December 28, 1912		$ 519.55

V. THE DIRECTOR'S REPORT.

To the Trustees of the Marine Biological Laboratory:

Gentlemen: I have the honor to transmit herewith a report of the twenty-fifth session of the Marine Biological Laboratory. The attendance of investigators at the Laboratory in 1912 exceeded that of previous years by a considerable number, 93 in 1912 as compared with 82 in 1911, and 62 in 1910. The number of students in the courses was 67 as compared with 65 in 1911 and 64 in 1910. The total attendance was 160 in 1912, 147 in 1911, and 126 in 1910. During part of the season the buildings were uncomfortably crowded; indeed if it had not been for the additional space furnished by the Kidder annex it would have been necessary to turn away a number of investigators. The student accommodations are at present ample and likely to remain so, but the limitation of space assignable to individual investigators, especially in the general research laboratories, is a serious handicap to the best kind of work. For the season of 1913 some additional space will be available, if needed, in the Yacht Club building; but the facilities in this building and in the Kidder annex will be at best makeshifts.

Fortunately, such conditions will not long continue, as the plans for the new laboratory building are now complete, and the building will be erected in the coming summer.

The number of subscribing institutions again showed an increase, from 25 in 1911 to 29 in 1912. In 1907 the number was 16 and the increase since has been steady. The following subscriptions were new: Carleton College, Else Seringhaus Scholarship of Normal College, Laboratory of Comparative Anatomy of Harvard Medical School, University of Texas, and Wabash College. It is gratifying to see this growing recognition by institutions of the value of the facilities offered by the Laboratory; but we must not cease to labor for its extension, because the Laboratory will be on a sounder basis when a larger number of institutions feel responsibility for their share of its support.

The growth of the Laboratory in numbers and the yearly purchase of houses in Woods' Hole by members of the Laboratory have combined to reduce the living accommodations in Woods Hole for transient workers at the Laboratory below the margin of safety. There is no doubt that if we have even a slight increase of numbers another year some will be unable to secure rooms in the village, and the price of available rooms will become excessive. Last season the Laboratory rented a house for use as a woman's dormitory with accommodations for about 14, and for some years past over 20 men have been accommodated in the stone building. We are face to face with the actual necessity of providing a dormitory for women. Some emergency provision must be made for the summer of 1913, and at the same time we must look beyond this to the necessity of more satisfactory and permanent provision. We cannot expect that the villagers will take care of any increase, because they find it more profitable to rent their houses to families than rooms to detached individuals.

After the erection of the new building it is possible that one of the smaller laboratory buildings might be made over as a dormitory for women; but whether this is desirable or not, it is important that the matter should receive prompt consideration in order that there may be no handicap on the attendance of women at the Laboratory. It is suggested that committees, both of the Board of Trustees and of the Corporation, be organized to consider the problem.

During the past summer the assistant director undertook to collect funds for much needed improvements and enlargement of the mess. Appeals were made to members of the Board of Trustees and of the Corporation and former members of the Laboratory with very gratifying results. There exists to-day, as in earlier periods of the history of the Laboratory, a strong feeling of loyalty and attachment to the interests of the institution, and it is my conviction that this feeling should be organized in some permanent form to serve definite needs of the Laboratory.

Care should be taken that no undue pressure be exerted, and that no one should come to feel his membership in the corporation in any sense a burden; but there can be no doubt that it is possible to avoid this and at the same time to secure effective support for various needs. Moreover, such activities heighten the sense of devotion of those taking part in them. Our organization is primarily coöperative; there should be adequate outlets for all who feel disposed to aid in any way.

Since the last annual report was made death has removed from our number Professor T. H. Montgomery, Jr., clerk of the corporation and ex officio a member of the board of trustees. At the summer meeting of the corporation and board of trustees of the Marine Biological Laboratory the following resolution was adopted:

"On March 19, 1912, Dr. Thomas Harrison Montgomery, Jr., professor of zoölogy in the University of Pennsylvania, clerk of the corporation and a trustee of the Marine Biological Laboratory, died at the early age of 39 years. He was a loyal friend of this Laboratory, with which he had been connected as investigator and instructor for about fifteen years. He was an investigator of high rank, whose discoveries have become a permanent part of biological science; an instructor and organizer of unusual ability; a man of fine instincts and lofty ideals, to whom the 'rare old name of gentleman' applied with peculiar force.

"The corporation and trustees of the Marine Biological Laboratory record their great loss and personal sorrow in his death, and extend to the members of his family deep sympathy in their bereavement."

Professor Montgomery's place in the corporation and board of trustees has been filled by the election of Dr. Gary N. Calkins, of Columbia University. Another vacancy in the board was filled by the election of Dr. H. H. Donaldson, of the Wistar Institute of Anatomy and Biology. To both of these gentlemen we extend out cordial greetings.

The treasurer's report shows a surplus of receipts over expenditures, thanks to the inexhaustible generosity of the president of the board, Mr. Crane. The running expenses were higher in 1912 than in 1911 owing to (1) the appointment of the assistant director to give full time to the Laboratory, (2) increase in the janitor and collecting service, (3) to slight unavoidable increases in the running expenses of various departments. The receipts from various sources have increased year by year, so that the relation of earned income to total running expenses has not varied much. The following table will be of interest in this connection:

Income.	1909	1910	1911	1912
1. Dues	$ 666.00	$ 606.00	$ 728.00	$ 716.00
2. Misc.	205.44	856.11	122.96	91.48 7.83
3. Supply dept.	8,549.55	9,300.58	10,303.61	13,966.35
4. Mess (net)		551.97	1,111.93	1,581.10
5. Tuition, etc.	3,708.35	4,150.00	4,574.99	5,175.00
Total	13,128.34	15,464.66	16,841.49	20,837.76
Expense, Total	21,585.79	22,547.48	27,929.07	31,526.46
Amount needed to be covered by donations.	8,457.45	7,082.82	11,087.58	10,688.70
Proportion of deficit to total expense	39+%	31+%	39+%	34−%

The total expenses outside of the actual purchase of real estate, building, and operation of the mess are given in these figures. The credits that might naturally be allowed for permanent improvements such as the building of an ice house and work shop and the purchase of the motor boat *Sagitta* in 1911 do not appear.

It is obvious that the expense of operation is rapidly increasing in correspondence partly with the growth in attendance and partly in consequence of the endeavor to furnish more efficient service. The estimates for 1913 show another probable increase of $4,000 to $5,000 in running expenses, and in 1914, with the new building in operation, yet farther increase is inevitable. The growth of the Laboratory entails increased financial responsibility. The only way of meeting the situation and proving equal to the opportunity of scientific service that is ours is to undertake at once to raise an endowment fund of not less than $500,000. With this to rely on the institution could hold permanently all that it has gained. Fortunately, the Laboratory has no debts, and an emergency could be met to a certain extent by radical retrenchment.

There are appended as parts of this report a list of the staff and of the students and investigators for 1912, together with a tabular view of attendance for the last five years; the list of subscribing institutions, the evening lectures, and the membership of the Corporation.

1. THE STAFF—1912

F. R. LILLIE, DIRECTOR,
Professor of Embryology, The University of Chicago.

GILMAN A. DREW, ASSISTANT DIRECTOR,
Marine Biological Laboratory.

ZOÖLOGY

I. INVESTIGATION

Zoölogy and Embryology

GARY N. CALKINS	Professor of Protozoölogy, Columbia University.
E. G. CONKLIN	Professor of Zoölogy, Princeton University.
GILMAN A. DREW	Assistant Director, Marine Biological Laboratory.
GEORGE LEFEVRE	Professor of Zoölogy, University of Missouri.
FRANK R. LILLIE	Professor of Embryology, The University of Chicago.
T. H. MORGAN	Professor of Experimental Zoölogy, Columbia University.
E. B. WILSON	Professor of Zoölogy, Columbia University.

II. INSTRUCTION

CASWELL GRAVE	Associate Professor of Zoölogy, Johns Hopkins University.
RAYMOND BINFORD	Fellow in Zoölogy, Johns Hopkins University.
A. S. PEARSE	Associate Professor of Biology, St. Louis University School of Medicine.
BERTRAM G. SMITH	Graduate Student of Zoölogy, Columbia University.
E. D. CONGDON	Instructor in Embryology, Cornell University Medical College.
ROBERT CHAMBERS, JR.	Graduate Student of Zoölogy, Columbia University.

EMBRYOLOGY

I. INVESTIGATION (See Zoölogy)

II. INSTRUCTION

Gilman A. Drew	Assistant Director, Marine Biological Laboratory.
Lorande L. Woodruff	Assistant Professor of Biology, Yale University.
William E. Kellicott	Professor of Biology, Goucher College.
Robert A. Budington	Associate Professor of Zoölogy, Oberlin College.

PHYSIOLOGY

I. INVESTIGATION

Albert P. Mathews	Professor of Physiological Chemistry, The University of Chicago.
R. S. Lillie	Instructor in Comparative Physiology, University of Pennsylvania.
Harold C. Bradley	Assistant Professor of Physiological Chemistry, University of Wisconsin.

II. INSTRUCTION

R. S. Lillie	Instructor in General Physiology, University of Pennsylvania.
Walter E. Garrey	Associate Professor of Physiology, Washington University Medical School.
Frank P. Knowlton	Professor of Physiology, Syracuse University.
Edward B. Meigs	Associate in Physiology, Wistar Institute of Anatomy and Biology.

PHILOSOPHICAL ASPECTS OF BIOLOGY AND ALLIED SCIENCES

LECTURES

Edward G. Spaulding	Assistant Professor of Philosophy, Princeton University.

BOTANY

George T. Moore	Professor of Plant Physiology and Applied Botany, Washington University.
George R. Lyman	Assistant Professor of Botany, Dartmouth College.
B. M. Duggar	Professor of Plant Physiology, Cornell University.

Ivey F. Lewis	Professor of Biology, Randolph-Macon College.
W. J. Robbins	Assistant in Plant Physiology, Cornell University.

LIBRARY

H. McE. Knower	University of Cincinnati, Librarian.

CHEMICAL SUPPLIES

Oliver S. Strong	College of Physicians and Surgeons, New York City, Chemist.

G. M. Gray	Curator of Supply Department.
Thomas M. Douthart and John J. Morton	Collectors in Zoölogy.
O. F. Curtis	Collector in Botany, Oberlin College.
John Veeder	Cockswain.

2. INVESTIGATORS AND STUDENTS 1912

INVESTIGATORS—1912

ZOOLOGY

Baitsell, George A., Assistant in Zoölogy, Yale University.
Beckwith, Cora J., Instructor in Biology, Vassar College.
Binford, Raymond, Fellow in Zoölogy, Johns Hopkins University.
Boring, Alice M., Assistant Professor of Zoölogy, University of Maine.
Budington, Robert A., Associate Professor of Zoölogy, Oberlin College.
Calkins, Gary N., Professor of Protozoölogy, Columbia University.
Cary, Lewis R., Instructor in Zoölogy, Princeton University.
Chambers, Robert, Jr., Associate Professor of Embryology and Histology, University of Cincinnati.
Clapp, Cornelia M., Professor of Zoölogy, Mount Holyoke College.
Clark, Eleanor Linton, Special Student, Johns Hopkins University.
Clark, Eliot R., Associate in Anatomy, Johns Hopkins University.
Conklin, Edwin G., Professor of Biology, Princeton University.
Dexter, J. S., Graduate Student, Columbia University.
Drew, Gilman, A. Assistant Director, Marine Biological Laboratory, Woods Hole, Mass.
Edwards, Dayton J., Tutor in Physiology, College of the City of New York.
Glaser, Otto C., Junior Professor of Zoölogy, University of Michigan.
Grave, Caswell, Associate Professor of Zoölogy, Johns Hopkins University.
Gregory, Louis H., Instructor in Zoölogy, Barnard College.
Harvey, E. Newton, Instructor in Zoölogy, Princeton University.
Hickernell, Louis M., Graduate Fellow, Princeton University.
Hogue, Mary J., Instructor in Zoölogy, Mount Holyoke College.
Kellicott, William E., Professor of Biology, Goucher College.
Knower, H. McE., Professor of Anatomy, University of Cincinnati.
Lewis, Margaret Reed, 1931 East 31st Street, Baltimore, Md.
Lewis, Warren H., Associate Professor of Anatomy, Johns Hopkins University.
Lillie, Frank R., Director, Marine Biological Laboratory, and Professor of Embryology, University of Chicago.
Loeb, Leo, Director, Department of Pathology, Barnard Free Skin and Cancer Hospital, St. Louis, Mo.
Lund, Elmer J., Bruce Fellow, Johns Hopkins University.
Malone, Edward F., Assistant Professor of Anatomy, University of Cincinnati.
Morrill, Charles V., Instructor in Anatomy, University and Bellevue Hospital Medical College, New York City.
Morgan, T. H., Professor of Experimental Zoölogy, Columbia University.
Nowlin, Nadine, Instructor in Zoölogy, University of Kansas.

Orcutt, Alfred W., Fellow in Zoölogy, University of Illinois.
Pappenheimer, Alwin M., Associate in Pathology, Columbia University.
Patterson, John T., Adjunct Professor of Zoölogy, University of Texas.
Pearse, A. S., Assistant Professor of Zoölogy, University of Wisconsin.
Smith, Bertram G., Assistant Professor of Zoölogy, State Normal College, Ypsilanti, Mich.
Spaulding, Edward G., Assistant Professor of Philosophy, Princeton University.
Stockard, Charles R., Professor of Anatomy, Cornell Medical College.
Strong, Oliver S., Instructor in Anatomy, Columbia University.
Wieman, H. L., Assistant Professor of Zoölogy, University of Cincinnati.
Williams, L. W., Instructor in Comparative Anatomy, Harvard Medical School.
Wilson, E. B., Professor of Zoölogy, Columbia University.
Woodruff, L. L., Assistant Professor of Biology, Yale University.

PHYSIOLOGY

Bancroft, Frank W., Associate, Rockefeller Institute for Medical Research.
Bradley, Harold C., Assistant Professor of Physiological Chemistry, University of Wisconsin.
Donaldson, H. H., Professor of Neurology, Wistar Institute of Anatomy and Biology.
Ewald, W. F., Fellow, Rockefeller Institute for Medical Research.
Garrey, W. E., Associate Professor of Physiology, Washington University Medical College.
Kite, George L., Fellow in Pathology, Sprague Institute, University of Chicago.
Knowlton, Frank P., Professor of Physiology, Syracuse University.
Lillie, Ralph S., Assistant Professor of Experimental Zoölogy, University of Pennsylvania.
Loeb, Jacques, Rockefeller Institute for Medical Research.
Lyon, E. P., Professor of Physiology, St. Louis University.
Meigs, Edward B., Fellow in Physiology, Wistar Institute of Anatomy and Biology.
Rosenow, Edward C., Assistant Professor of Medicine, Rush Medical College.
Wasteneys, Hardolph, Associate, Rockefeller Institute for Medical Research.
Wherry, William B., Professor of Bacteriology, Cincinnati Hospital, Cincinnati, Ohio.

BOTANY

Derick, Carrie M., Professor of Morphological Botany, McGill University.
Duggar, Benjamin M., Professor of Plant Physiology, Washington University.
Fromme, Fred. D., Assistant in Botany, Columbia University.
Harper, Robert A., Professor of Botany, Columbia University.
Lewis, Ivey F., Professor of Biology, Randolph-Macon College.
Lyman, George R., Assistant Professor of Botany, Dartmouth College.
Marquette, William G., Columbia University.
Moore, George T., Director, Missouri Botanical Gardens, St. Louis.
Osterhout, W. J. V., Assistant Professor of Botany, Harvard University.
Robbins, William J., Assistant in Plant Physiology, Cornell University.

BEGINNING INVESTIGATORS

ZOÖLOGY

Adkins, Walter S., Graduate Student, Columbia University.
Allyn, Harriet M., Instructor in Zoölogy, Vassar College.
Browne, Ethel N., Research Assistant, Princeton University.
Carver, Gail C., Graduate Student, Columbia University.
Dungay, Neil S., Graduate Student, University of Chicago.
Flanigen, Ruth, Woodbury, New Jersey.
Goodrich, Hubert B., Assistant in Zoölogy, Columbia University.
Hayden, Margaret A., Teacher of Biology, Western High School, Baltimore, Md.
Heilbrunn, Lewis V., Graduate Student, Columbia University.
Hewitt, Joseph H., 2145 North Halsted Street, Chicago, Ill.
Hoge, Mildred A., Graduate Student, Columbia University.
Howland, Ruth B., Associate Professor of Biology, Sweet Briar College.
Just, Ernest E., Associate Professor of Biology, Howard University.
McCann, William S., Graduate Student, Cornell Medical College.
Morris, Margaret, 53 Edgehill Road, New Haven, Conn.
Packard, Charles, Assistant in Zoölogy, Columbia University.
Patten, Hazel, Laboratory Assistant, Western High School, Baltimore, Md.
Sink, Emory W., Assistant in Zoölogy, University of Michigan.
Spencer, Henry J., Graduate Student, Columbia University.
Wallace, Edith M., Research Assistant, Columbia University.
Wheeler, Isabel, Graduate Student, Columbia University.

PHYSIOLOGY

Kanda, Sakyo, Fellow in Psychology, Clark University.
Kellersberger, Eugene R., Graduate Student, Washington University Medical School.

BOTANY

Bartholemew, Elbert T., Instructor in Botany, University of Wisconsin.
Howe, Caroline G., Teacher of Biology, East Side High School, Newark, N. J.

STUDENTS

1912

ZOÖLOGY

Adams, Ethel M., Assistant in Biology, Carleton College, Northfield, Minn.

Allard, Anne D., Teacher, Boston Normal School, Boston, Mass.

Behm, Florence C., Assistant in Biology, Syracuse University.

Brooks, Alice D., Mount Holyoke College.

Clark, Katharine, Oberlin College.

Cogswell, Marguerite A., Instructor in Biology, The Misses Maxen's School, Dobbs Ferry, New York.

Ferguson, Russell S., Student, University of Maine.

Frank, Mrs. Simon W., 22 Talbot Road, Windsor Hills, Baltimore, Md.

Gover, Mary, 600 North Carrollton Avenue, Baltimore, Md.

Griest, Ellwood, Assistant in Zoölogy, University of Michigan.

Halsted, Halcyon, Student, College of Physicians and Surgeons, Columbia University.

Hedge, Mabel L., Research Assistant in Zoölogy, Columbia University.

Illick, J. Theron, Graduate Student, Syracuse University.

Johnson, Edith M., 150 N. 20th Street, Philadelphia, Pa.

Kennerly, Martha M., Instructor, Normal College, New York City.

Lambert, Robert A., Associate in Pathology, Columbia University.

McDonald, J. Daly, Oberlin College.

Mackenzie, John A., Student, Williams College.

Matlock, Charlotte L., Woodbury, New Jersey.

Pasternak, Jesse, 303 President Street, Brooklyn, N. Y.

Renfrew, Franklin W., 177 Woodruff Avenue, Brooklyn, New York.

Spence, Elizabeth, Student, University of Chicago.

Tredick, Helen F., Teacher of Biology, Erasmus Hall High School, Brooklyn, New York.

White, Edith G., 77 Brighton Avenue, Allston, Mass.

EMBRYOLOGY

Bradley, Barbara, 62 Trumbull Street, New Haven, Conn.

Cashman, Marie E., Graduate Student, Syracuse University.

Dolly, William Lee, Jr., Student Assistant, Johns Hopkins University.

Ferguson, Albert V., Student, University of Maine.

Ickes, Marguerite, The Normandie, Columbus, Ohio.

Isaacs, Raphael, Assistant in Zoölogy, University of Cincinnati.

Jameson, A. Pringle, Assistant in Zoölogy, University of Aberdeen, Aberdeen, Scotland.

Jarvis, Marjorie M., Student Assistant, University of Texas.

Paige, Beryl H., Laboratory Assistant, Mount Holyoke College.

Root, Francis M., 150 N. Professor Street, Oberlin, Ohio.
Schlehner, H. W., Graduate Student, University of Pennsylvania.
Shaw, Mary B., Barnard College, Columbia University.
Stark, John R., 110 S. McMillan Street, Cincinnati, Ohio.
Surls, Joseph K., Student, Williams College.
Wilson, Charlotta W., 103 LeMoyne Avenue, Washington, Pa.

PHYSIOLOGY

Clapp, G. A., Assistant in Zoölogy, Oberlin College.
Elkus, Savilla A., Instructor in Philosophy, Vassar College.
Field, Hazel E., Teacher of Science, Belhaven, Jackson, Miss.
Goldman, Irma A., Student, University of Kansas.
May, Henry G., Student, University of Rochester.
Oppenheimer, Adele, Volunteer, Pathology Laboratory, College of Physicians and Surgeons, Columbia University.
Overton, James B., Associate Professor of Plant Physiology, University of Wisconsin.
Spaeth, Reynold A., Teaching Fellow in Zoölogy, Harvard University.
Stewart, Norman H., Assistant Professor of Biology, Bucknell University.
Wardwell, Edward H., M. E. Fellow in Biology, Princeton University.
Wislocki, George B., Student, Washington University, St. Louis, Mo.

BOTANY

Benedict, Don M., Portland, Michigan.
Curtis, Otis F., 103 North Main Street, Oberlin, Ohio.
Duff, Dorothy, Student, McGill University.
Ewing, Arthur E., 5956 Cabanne Place, St. Louis, Mo.
Foster, Goodwin L., Student, Dartmouth College.
Gustafson, Felix, Student, Northland College, Ashland, Wisconsin.
Hoar, Carl S., Graduate Student, Harvard University.
Hopping, Aleita, Student, Normal College, New York City.
LeVan, William C., Catawissa, Pa.
Miller, Clare, Student, McGill University.
Noble, Arlyle, Smith College, Northampton, Mass.
Pennypacker, John Y., Instructor in Biology, Central High School, Philadelphia, Pa.
Stone, Mabel A., Instructor in Botany, Wellesley College.
Thomas, Cecil C., Instructor in Botany, Wabash College.
Weeks, Lucy M., Student, Oberlin College.
Weston, William H., Jr., Graduate Student, Harvard University.
Wood, Isabelle T., Student, Vassar College.

3. TABULAR VIEW OF ATTENDANCE

	1908	1909	1910	1911	1912
Investigators—Total	52	66	62	82	93
Occupying Rooms					
Zoölogy	32	39	33	42	44
Physiology	6	7	11	18	14
Botany	8	4	9	8	10
Occupying Tables					
Zoölogy	6	14	6	12	21
Physiology		2	2	2	2
Botany					2
Students—Total	48	63	64	65	67
Zoölogy	19	36	31	26	24
Embryology	15	12	10	20	15
Physiology	3	9	5	6	11
Botany	11	6	17	13	17
Total Attendance	100	129	126	147	160
Institutions Represented—Total					57
By Investigators	25	27	26	37	43
By Students	26	20	24	31	36
Schools and Academies Represented					
By Investigators	2	3	5	3	2
By Students	3	11	6	9	1

4. SUBSCRIBING INSTITUTIONS, 1912

Carleton College.
Columbia University.
Dartmouth College.
Else Seringhaus Scholarship of Normal College, New York.
Goucher College.
Harvard Medical School, Laboratory of Comparative Anatomy.
Lucretia Crocker Scholarship—Boston Public Schools.
Milton Hersey Scholarship—McGill University.
Mount Holyoke College.
Oberlin College.
Princeton University.
Rockefeller Institute for Medical Research.
Sheffield Scientific School, Yale University.
Smith College.
Syracuse University.
University of Chicago.
University of Cincinnati.
University of Illinois.
University of Kansas.
University of Michigan.
University of Pennsylvania.
University of Rochester.
University of Texas.
Vassar College.
Wabash College.
Western College for Women.
Wellesley College.
Williams College.
Wistar Intsitute of Anatomy and Biology.

5. EVENING LECTURES, 1912

Prof. W. E. Castle	"Unit Characters in Heredity"	Friday, July 5.
Prof. Charles S. Minot	"Senescence"	Tuesday, July 9.
Prof. Frank M. Chapman	"A Natural History Reconnaissance in Mexico"	Friday, July 12.
Dr. George H. Shull	"The Bearing of Cross and Self-Fertilization on Heredity and Evolution"	Tuesday, July 16.
Prof. H. E. Crampton	"A Biological Reconnaissance in South America,"	Friday, July 19.
Dr. R. S. Lillie	"The Role of Membranes in Cell-Processes"	Tuesday, July 23.
Dr. C. M. Child	"Certain Dynamic Factors in Reproduction and their Significance"	Friday, July 26.
Prof. E. G. Conklin	"The Mechanism of Cell-Division"	Tuesday, July 30.
Prof. W. L. Tower	"Experimental Transmutation of Specific and Generic Characters"	Friday, Aug. 2.
Prof. E. O. Jordan	"The Spread of Typhoid"	Tuesday, Aug. 6.
Prof. Charles E. Bessey	"The Phyletic Arrangement of Flowering Plants"	Friday, Aug. 9.

6. MEMBERS OF THE CORPORATION

LIFE MEMBERS, 1912

ALLIS, MR. E. P., JR., Palais Carnoles, Menton, France.
ANDREWS, MRS. GWENDOLEN FOULKE, 821 St. Street, Baltimore, Md.
BILLINGS, MR. R. C., 66 Franklin Street, Boston, Mass.
CAREY, MR. ARTHUR ASTOR, Fayerweather Street, Boston, Mass.
CLARKE, PROF. S. F., Williams College, Williamstown, Mass.
CONKLIN, PROF. EDWIN G., Princeton University, Princeton, N. J.
CRANE, MR. C. R., 2559 Michigan Boulevard, Chicago, Ill.
DAVIS, MAJOR HENRY M., Syracuse, New York.
EVANS, MRS. GLENDOWER, 12 Otis Place, Boston, Mass.
FARLOW, PROF. W. G., Harvard University, Cambridge, Mass.
FAY, MISS S. B., 88 Mt. Vernon Street, Boston, Mass.
FOLSOM, MISS AMY, 88 Marlboro Street, Boston, Mass.
FOOT, MISS KATHARINE, 80 Madison Avenue, New York City.
GARDINER, MRS. E. G., Woods Hole, Mass.
GARDINER, MISS EUGENIA, 15 West Cedar Street, Boston, Mass.
HANNAMAN, MR. CHARLES E., 103 First Street, Troy, New York.
HARRISON, EX-PROVOST C. C., University of Pennsylvania, Philadelphia
HIGGINSON, MR. HENRY L., 191 Commonwealth Avenue, Boston, Mass.
JACKSON, MISS M. C., 88 Marlboro Street, Boston, Mass.
JACKSON, MR. CHARLES C., 24 Congress Street, Boston, Mass.
KENNEDY, MR. GEORGE G., 284 Warren Street, Roxbury, Mass.
KIDDER, MR. C. G., 27 William St., New York City.
KIDDER, MR. NATHANIEL T., Milton, Mass.
KING, MR. CHARLES A.
LEE, MRS. FREDERIC S., 279 Madison Avenue, New York City.
LOWELL, MR. A. LAWRENCE, 171 Marlboro St., Boston, Mass.
MARRS, MRS. LAURA NORCROSS, 9 Commonwealth Avenue, Boston, Mass.

Mason, Miss E. F., 1 Walnut Street, Boston, Mass.
Mason, Miss Ida M., 1 Walnut Street, Boston, Mass.
Means, Mr. James Howard, 196 Beacon Street, Boston, Mass.
Merriman, Mrs. Daniel, Worcester, Mass.
Minns, Miss Susan, 14 Louisburg Square, Boston, Mass.
Minns, Mr. Thomas, 14 Louisburg Square, Boston, Mass.
Minot, Dr. Charles S., Harvard Medical School, Boston, Mass.
Mixter, Miss M. C., 241 Marlboro Street, Boston, Mass.
Morgan, Mr. J. Pierpont, Jr., Wall and Broad Sts., New York.
Morgan, Prof. T. H., Columbia University, New York City.
Morgan, Mrs. T. H., New York City.
Noyes, Miss Eva J., 28 South Willow St., Montclair, N. J.
Nunn, Mr. Lucian L., Telluride, Colo.
Osborn, Prof. Henry F., American Museum of Natural History, New York.
Pell, Mr. Alfred, Highland Falls, Orange Co., N. J.
Phillips, Dr. John C., 299 Berkeley St., Boston, Mass.
Phillips, Mrs. John C., 299 Berkeley Street, Boston, Mass.
Porter, Dr. H. C., University of Pennsylvania, Philadelphia, Pa.
Pulsifer, Mr. W. H., Newton Center, Mass.
Rogers, Miss A. P., 5 Joy Street, Boston, Mass.
Sears, Dr. Henry F., 420 Beacon St., Boston, Mass.
Shedd, Mr. E. A.
Smith, Mrs. C. C., 286 Marlboro St., Boston, Mass.
Strobell, Miss E. C., 80 Madison Avenue, New York City.
Thorndike, Dr. Edward L., Teachers' College, Columbia University, New York City.
Trelease, Prof. William, Missouri Botanical Gardens, St. Louis, Mo.
Ware, Miss Mary L., 41 Brimmer Street, Boston, Mass.
Warren, Mrs. S. D., 67 Mt. Vernon St., Boston, Mass.
Whitney, Mr. Henry M., Brookline, Mass.
Willcox, Miss Mary A., Wellesley College, Wellesley, Mass
Wilmarth, Mrs. H. D., Elliott Street, Jamaica Plain, Mass.
Williams, Mrs. Anna P., 505 Beacon Street, Boston, Mass.
Wilson, Dr. E. B., Columbia University, New York City.
Wilson, Prof. W. P., Philadelphia Museum, Philadelphia, Pa.

MEMBERS, AUGUST, 1912

ABBOTT, PROF. J. F., Washington University, St. Louis, Mo.

ABBOTT, MARGARET B., Bennett School, Millbrook, New York.

ADAMS, DR. C. F., University of Arkansas, Fayetteville, Ark.

ADDISON, DR. W. H. F., University of Pennsylvania, Philadelphia, Pa.

ADKINS, MR. W. S., Department of Zoölogy, Columbia University, New York.

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